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UNIVERSITY. OF ALBERTA

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SOME STORAGE CHARACTERISTICS OF CHEMICALLY TREATED HIGH MOISTURE GRAIN

by

© IVAN TERRENCE KOZMECH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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EDMONTON, ALBERTA

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Some Storage Characteristics of Chemically Treated High Moisture Grain" submitted by Ivan Terrence Kozmech in partial fulfilment of the requirements for the degree of Master of Science.

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Date 11th September, 1973

ABSTRACT

The principal objective of this project was to investigate the storage characteristics of high moisture feed barley treated with marginal Chemstor application rates. Samples in replicates of two were conditioned to an average moisture content of 18.7, 22.6 and 25.0%. Subsamples of each moisture group were sprayed with Chemstor at an overall average of 29, 41 and 51% of the manufacturer's recommended rate. The samples were subdivided into units of 130 grams and stored in Erlenmeyer flasks at the experimental temperatures of 12.5, 28.0 and 37.0°C.

The samples were periodically analyzed for carbon dioxide production. An analysis for mould and bacteria was conducted at 50, 100 and 150 days. Acidity values were recorded at the commencement and termination of the project.

The data obtained showed that:

1. The predominate moulds isolated were *Aspergillus ruber*, *Aspergillus glaucus* and *Aspergillus flavus*. The relative abundance of each mould was influenced by the Chemstor application rate, moisture content and temperature.
2. *Mucor*, *Penicillium* and *Cladosporium* species were isloated in trace quantities.
3. The application of Chemstor at 41% and 51% of the commercially recommended rate maintained the keeping quality of the grain effectively for up to the experimental period of 150 days.

4. Barley stored at 37.0°C exhibited a higher total acidity content then that stored at 12.5°C or 28.0°C.

5. Virtually no moulds or bacteria were isolated at a storage temperature of 37.0°C regardless of the Chemstor application rate. Mould and bacterial counts were obtained at 12.5°C and 28.0°C storage temperatures with greater numbers occurring at the lower temperature.

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1. INTRODUCTION

1.1 Purpose

The feeding of grain to beef and dairy animals provides a means of indirectly marketing grain. Both dry and high moisture grain[a,b] can be processed by grinding or crushing, and this product used as an animal feed. In Western Canada, dry grain is normally processed; however, this may change since reports show that animals obtain a better feed conversion efficiency when fed high moisture grain rather than dry grain (16).

Unless unfavorable weather conditions result in the harvesting and storage of high moisture grain, the grain commonly will be harvested in a dry condition, stored and rewetted prior to processing. Rewetting is an unnecessary step if high moisture grain can be harvested and stored successfully without the fear of heating, spoilage and deterioration by microflora. The ecological relationships of these microflora, comprised of moulds, bacteria, yeasts and

---

a. According to the current grading system (30), cereal grain is divided into three categories on the basis of its moisture content. Dry grain is grain with a moisture content up to 14.5%. Tough grain is one with a moisture content between 14.5 and 17.0%. When the moisture content is above 17.0%, grain is graded as damp. In this experiment, grain containing more than 17.0% moisture is considered to be high moisture grain.

b. In this thesis, all moisture contents are given on a wet weight basis.

actinomycetes, are complex. The microbial activities are influenced by the moisture and temperature of the stored grain. Low temperatures offset the effect of high moisture with respect to microbial development and deterioration.

Microflora are not the only cause of high moisture grain deterioration. Biochemical changes may cause seed discoloration, reduced germination, and an increase in the fatty acid value. These changes can result in a condition known as 'sick' grain. Although the deterioration process is slow, it is favored by high moisture and temperature.

During the 1960's, liquid grain preservatives were successfully used to prevent microbial deterioration of high moisture grain. The chemicals involved were mainly the lower fatty acids, namely, propionic acid, acetic acid and various mixtures of these acids. The use of these chemicals is restricted to grain used for animal feed. The propionic and acetic acids are naturally occurring acids associated with ruminant metabolism of dairy and beef animals (17), therefore, chemically treated high moisture grain is not hazardous to the animals.

In Alberta, Canada, a commercial preservative under the trade name of CHEMSTOR (60% acetic acid and 40% propionic acid by volume) can be used to preserve high moisture feed grain. The manufacturer of Chemstor claims that this preservative, when applied at their recommended rates, will provide indefinite storage of high moisture feed grains.

Two factors, namely, moisture content and temperature of the grain entering storage, influence the effectiveness of Chemstor. The constituents of Chemstor are more effective at higher temperatures, but

less effective at higher moistures. Since increases in grain moisture are associated with increases of Chemstor application rates, the manufacturer has based these rates on the moisture content of the grain and not temperature (7).

Since feed grains are consumed by animals daily, the treatment of feed grains for indefinite storage is not necessary. Tests conducted in England and Sweden show promising results for short term ambient storage of high moisture grain treated with reduced or marginal levels of chemical preservative (31,37,38).

In this experiment, an attempt was made to simulate and study the effect of variations in storage temperature of the grain, moisture content of the grain, and the marginal application rates of Chemstor upon the microfloral ecology and the development of 'sick' grain in high moisture grain stored in Alberta. The concept of carbon dioxide (CO_2) production, a by-product of respiration, was used as an index of microbial development. According to the manufacturer, Chemstor not only inhibits or kills microflora, but also sterilizes the grain kernels (7). A substantial increase in CO_2 produced can serve as an indicator of microbial growth.

1.2 Objectives

The objectives of this study were to:

1. simulate the moisture content and temperature at which high moisture barley may enter storage,
2. simulate the temperature changes that may occur in grain during storage,
3. investigate the effect of temperature on marginal levels of Chemstor,

4. quantify the bacteria and moulds on the treated high moisture barley,
5. identify the prevailing mould species, and
6. study the effect of temperature and length of storage on the development of 'sick' grain.

2. LITERATURE REVIEW

2.1 Respiration of grain.

Grain is a biologically living material which continues to respire when placed in storage. The rate of respiration, which is attributable to the metabolism of the grain kernels, the associated microbial population and, to a lesser extent, of insects, is influenced by moisture content, temperature, and oxygen (O_2) and CO_2 concentration of the intragranular atmosphere.

2.1.1 Influence of moisture content.

The respiration rate of dry grain is very low. However, the respiration rate increases as the moisture content of the grain increases. Bailey (3) found:

1. that increases in the respiration of grain occurred at a rather constant relative humidity of 75%, and
2. that different grains have different critical moisture values.

Thus, the critical value for wheat appeared to be about 14.6% and for barley 14.5%.

More recently, Hyde (19) has shown that the rate of respiration of microbial-free grain increases relatively little with increasing moisture content. The results indicated that the grain contributed very little to the total respiration - approximately 5.0 milligrams CO_2/100 grams dry matter/24 hours.

2.1.2 Influence of temperature

An increase in temperature accelerates respiration and a decrease in temperature limits respiration. Milner and Geddes (29) stated that the normal rate of respiration of grain may not be established

for several days since the seeds release CO_2 when wetted or their temperature elevated. The initial CO_2 output ceases in 24 to 48 hours and normal respiration then ensues, whether respiration be due to the seed itself or to fungal activity.

Many investigators have used CO_2 output as the only indication of respiration attributed to grain; no distinction was made to respiration attributed to microorganisms and to that of the grain kernels. Bailey and Gutjar (4), for example, studied the respiration of 15.0% moisture hard red spring wheat sealed in jars for four days, and concluded that the CO_2 output reached a maximum at 55.0°C. Milner and Geddes (27) studied the influence of temperature on respiratory activity of 18.0% moisture soybeans, between 25.0 and 45.0°C, and concluded that the optimum CO_2 output occurred at 40.0°C.

2.1.3 Influence of oxygen and carbon dioxide concentrations.

The O_2 and CO_2 concentration of the intragranular atmosphere affects the rate of respiration. An ambient CO_2 concentration of 12% suppressed the respiration of soybean and wheat to about 85% of that in air (26,27). Respiration occurs at many concentrations of these gases. The breakdown of carbohydrates and fats yielding CO_2 and water is oxidative or aerobic respiration. The fermentative process (anaerobic respiration) in the absence of O_2 results in CO_2, ethyl alcohol and various acids (29).

2.1.4 Measurement of respiration.

Two general methods are employed to measure the CO_2 production of grain. In one, the grain is kept in a closed container and the atmosphere analyzed for its CO_2 content at the termination of

the trial. In the second method, the container is aerated continuously or intermittently, thereby enabling periodic analysis of the atmosphere for its CO_2 content. Most commonly, humidified CO_2-free air is passed through the container holding the sample, and the CO_2 produced is absorbed in an alkali solution and then titrated with an acid using a color indicator. The results are normally expressed as:

- milligrams CO_2/100 grams dry matter/24 hours.

2.2 Temperature variations of bulk grain.

Temperature variations in stored bulk grain may be attributed to the initial grain temperature entering storage and the influence of external temperatures.

2.2.1 Initial grain temperature.

During the fall season when the grain is being harvested, the ambient air temperature can vary considerably from early morning to late evening, thus affecting the temperature at which grain enters storage. Furthermore, some grain enters storage above ambient temperatures due to radiant energy. Williamson (43) measured temperatures of grain harvested in sunny weather and found grain bulk temperatures 10-16^{o}F above the ambient.

2.2.2 Influence of external temperatures.

The external sources of heat are due to the daily and seasonal temperature fluctuations. Babbit (2) made estimates from mathematical formulae for variations in the temperatures of wheat stored in unventilated grain elevators. The results indicated that the effect of daily temperature variations was scarcely noticeable below five inches; the annual temperature variations of grain at a depth of 13 feet was never greater than 1^{o}F; and temperatures greater than 20

feet from the surface were practically unchanged and the wheat remained at the temperature of storage.

2.3 Moisture variation of bulk grain.

Moisture variations in stored bulk grain may be attributed to the initial moisture content of the grain entering storage and to moisture migration that can occur during storage.

2.3.1 Moisture content of bulk grain.

The equilibrium moisture content of the grain kernels varies according to the temperature and relative humidity of the surrounding air (figure 1). The relative humidity of air has a diurnal range, changing from hour to hour, and also a day-to-day variation (figure 2). The relative humidity is generally at a maximum when the air temperature is at its lowest, around dawn, and at a minimum about mid-afternoon when the air temperature is highest. Therefore, during a harvesting day, grain of varying moisture content may be placed into storage. Moisture redistribution occurs within bulk stored grain. Moisture from kernels of high moisture content migrates to kernels of lower moisture content until equilibrium is reached between kernels.

2.3.2 Moisture migration.

Hall (13) states that a safe moisture content does not necessarily guarantee safe storage in bulk. During the fall warm grain may be placed into storage. The grain mass will cool at the periphery but not appreciably in the centre. As a result, convection currents are set up whereby warm air from the centre of the bin meets cool damp atmospheric air at the top of the grain mass, resulting in moisture condensation (figure 3). In the spring there is a reversal

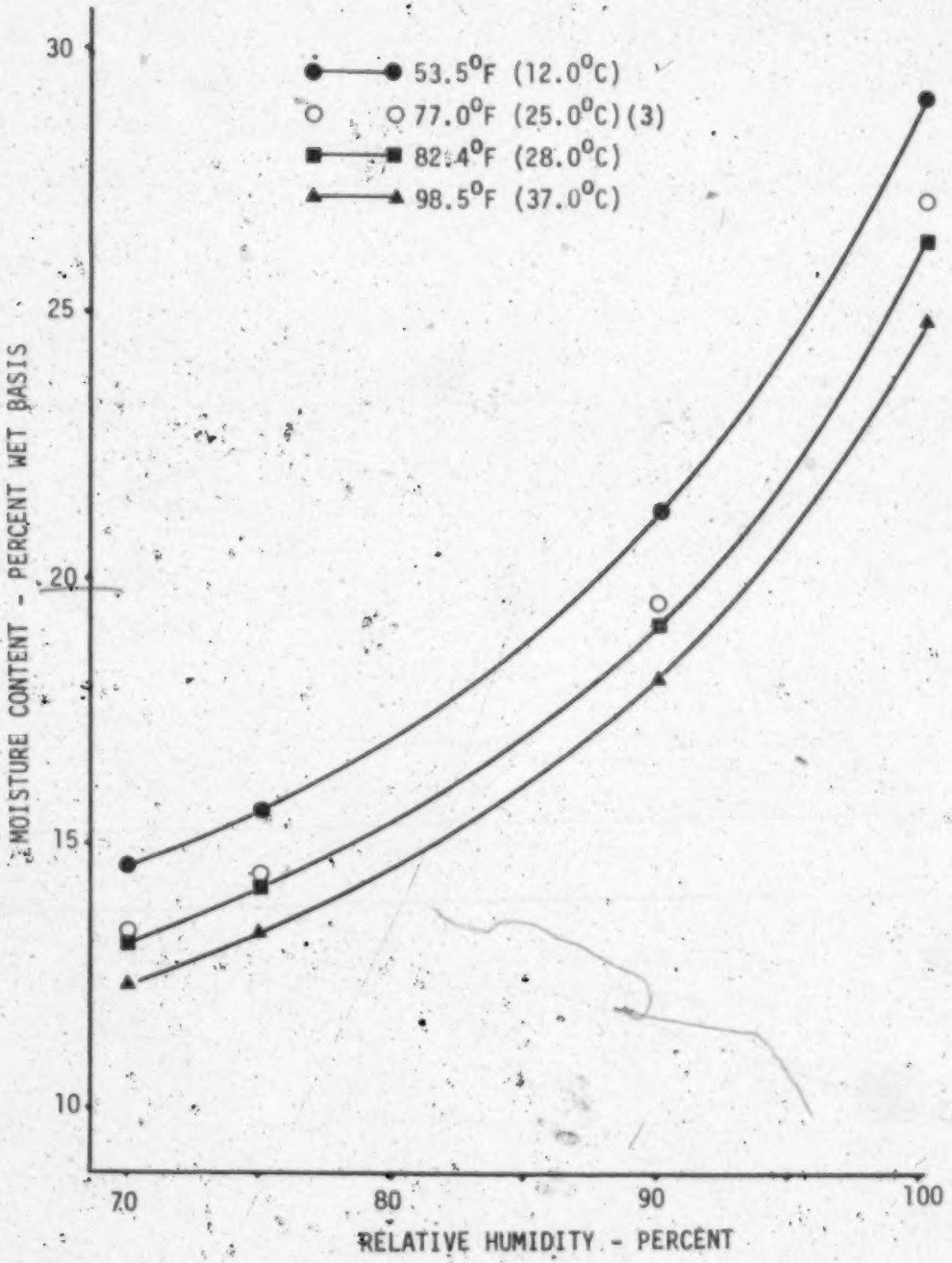

Figure 1: Moisture content and relative humidity relationship of barley at different temperatures (19).

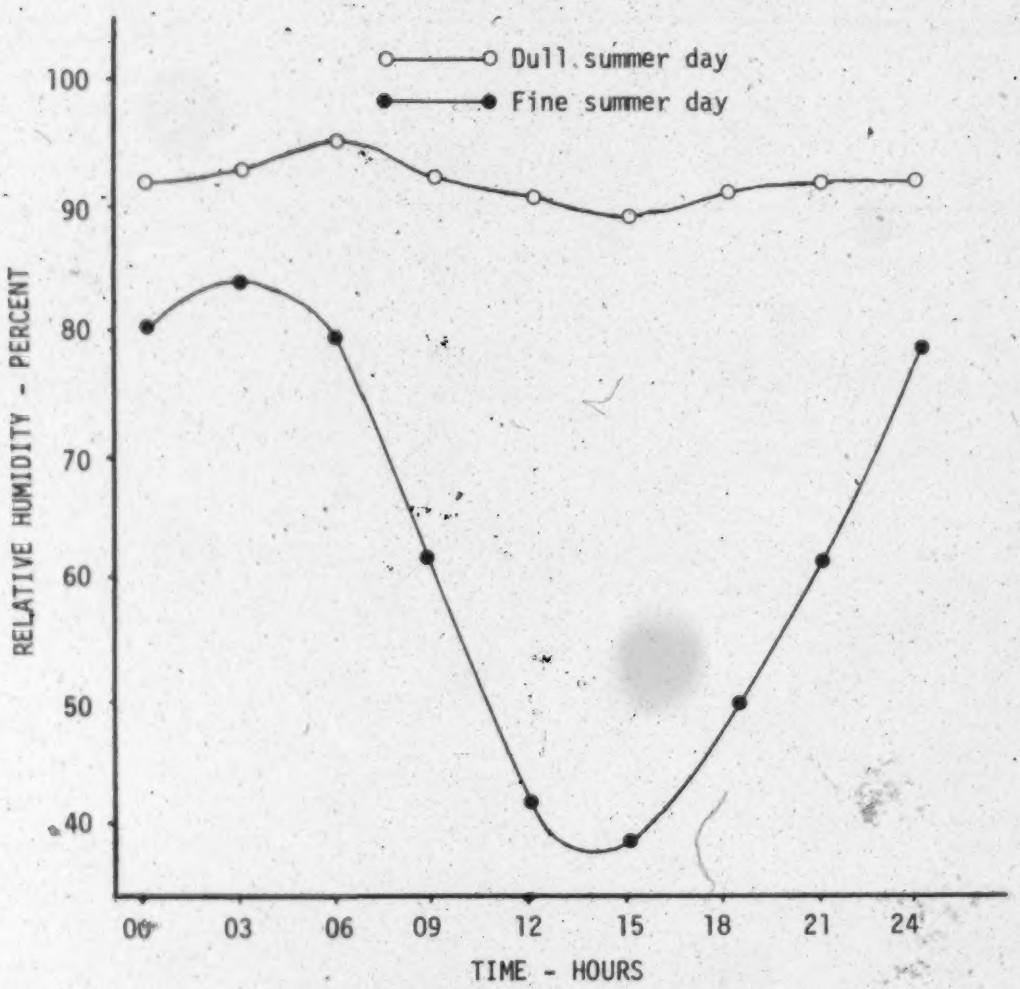

Figure 2: Diurnal variation of relative humidity (11).

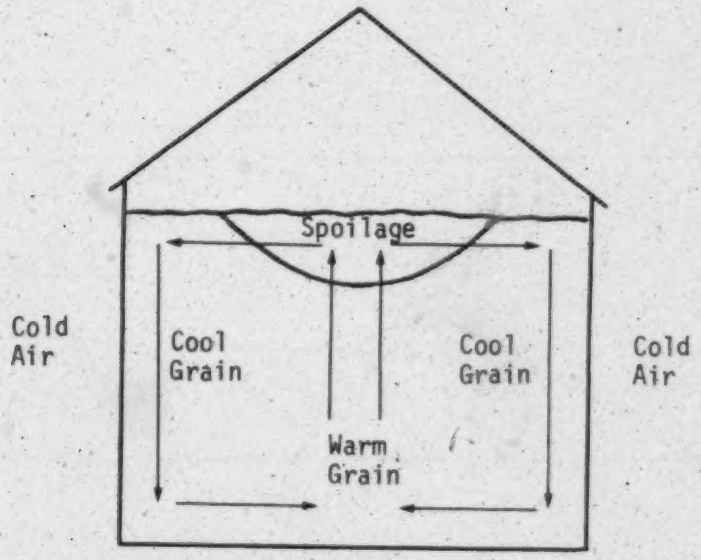

Figure 3: Convection currents with warm grain and cool atmospheric air (13).

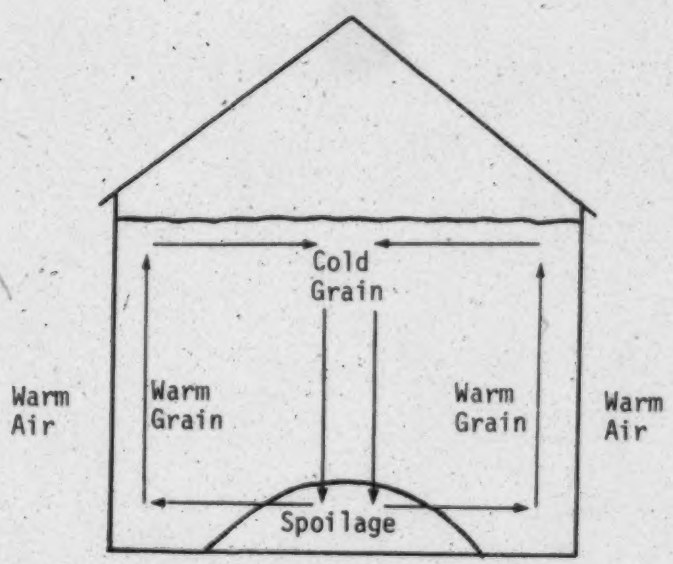

Figure 4: Convection currents with cool grain and warm atmospheric air (13).

of this moisture distribution and an area of spoilage may occur at the bottom of the bin (figure 4).

2.4 Microflora of grains.

Microorganisms are present on the outside and inside of all grains. Their abundance on the grain kernels depends upon the soundness of the kernels, the location and condition of harvest, and the extent of microbial invasion.

The moulds and bacteria which may develop in stored grain not only consume its valuable nutritive components but may be a hazard to the health of both humans and animals.

2.4.1 Abundance of microflora.

Bacteria outnumber moulds on a per weight basis as bacteria are present during all stages of grain development. Numerous investigators have reported the abundance of bacteria and moulds on grain. Semeniuk (34) summarized the results of the investigators as follows:

1. the number of bacteria varied from 8,000 to 12,000,000 per gram.
2. the number of moulds present on grain products varied from
   - (i) 98 to 920 per gram of wheat,
   - (ii) 0 to 440,000 per gram of Russian or German wheat, oats and barley, and
   - (iii) 420 to 1,879 per gram of Red Canadian Spring Wheat.

2.4.2 Types of microflora.

Christensen (8) states that the fungi in seeds may be divided into two groups, namely, field fungi and storage fungi. The field fungi are those that invade the developing or mature seed while it is still on the plant. The common genera are *Alternaria*, *Helminthosporium*, *Fusarium*, *Rhizopus*, *Absidia*, and *Cladosporium*. These fungi, with the possible exception of *Fusarium* (8), do not cause deterioration of stored grain.

The storage fungi are those which develop on and within the seeds and are principally the *Aspergillus* and *Penicillium* species. The ecology and relative abundance of these species depends upon their optimum and limiting moisture and temperature conditions. Thus, the *Aspergillus glaucus* group is the major fungi which invade 13.0 to 15.0% moisture grain and produce water as a by-product of growth. An increase in moisture content to above 15.5% allows the *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus candidus* groups to appear. Subsequent increases in moisture above 18.0% allows *Penicillium* and other groups of *Aspergillus* to appear and proliferate (8,31,35).

2.4.3 Water and temperature requirements of moulds.

Table 1 gives the optimum and limiting temperature and moisture conditions for some *Aspergillus* and *Penicillium* species.

2.5 Biochemical changes in stored grain.

Deterioriation in stored grain alters the fat acidity, phosphate acidity and amino acid acidity which, when combined, equal the total acidity. The fat acidity or fatty acid value (FAV) increases at a greater relative rate than the other types of acidity and, therefore, is commonly used as an index of grain deterioriation (46).

TABLE 1: APPROXIMATE TEMPERATURE AND MOISTURE REQUIREMENTS OF THE *Aspergillus* AND *Penicillium* SPECIES.

	Optima		Limits	
	Temperature °C	Water Activity+	Temperature °C	Water Activity+
A. *repens**	24	0.93	7 38	0.71
A. *chevalieri**	33	0.93	10 42	0.71
A *nidulans**	37	0.97	12 47	0.78
A. *flavus**	33	0.98	12 43	0.78
A. *fumigatus**	40	0.97	12 53	0.82
A. *candidus***	32	0.98	10 44	0.75
P. *martensii**	23	0.98	5 32	0.79
P. *islandicum**	31	0.97	10 38	0.83
P. *cyclopium**	23	0.98	5 32	0.83
A. *ruber***	24	0.93	5 38	0.72
A. *niger***	35	0.99	10 45	0.17

* Data from Ayerst (1).

** Data from Hall (14).

\+ water activity - ratio of the vapor pressure of the water in a media to that of pure water at the same temperature and pressure. When expressed as a percent, the water activity is referred to as the equilibrium relative humidity.

Carter and Young (6) produced 'sick' grain by storing Fulcaster wheat in sealed containers at various levels of moisture and temperature over time intervals up to 687 days. By arbitrarily eliminating all samples on which mould growth was visible, they concluded that 'sick' wheat formation was associated with anaerobic storage.

Milner et al (25) stored wheat for periods of up to six months at varying moisture contents under inhibiting and non-inhibiting mould atmospheres. They found that the mould-inhibited wheat became 'sick' to the same extent as that under non-inhibited conditions.

In other investigations, Milner et al (26) and Milner and Geddes (28) compared the FAV increases between dry grain, inhibited high moisture grain and non-inhibited high moisture grains. Since the trials were conducted at one temperature over a short time interval, the results did not show the influence of temperature and duration of storage. For example, soybeans were stored at 37.8^{o}C for 15 days and Regent wheat at 30.0^{o}F for 17 days. From the results, they concluded that there were slight changes in the chemical composition between dry grain and inhibited high moisture grain but that there were substantial increases in the FAV in the high moisture non-inhibited grain.

These investigations indicated that the effect of mould growth was additive to the other deteriorative processes responsible for 'sick' grain.

2.6 Chemical preservation.

The use of organic acids such as acetic and propionic acids, and their respective salts is not a recent innovation in food preservation. For example, in 1906 acetic acid was found to inhibit a

disease of bread dough called 'rope' (40). However, the researchers were more concerned with the maximum bacteriostatic action on the pathogenic organisms and minimum toxicity to humans rather than prolonged food preservation.

2.6.1 Mode of action.

In reviewing the literature, the consensus of researchers favor the theory that the undissociated molecules of the propionic and acetic acid are responsible for microbial toxicity. Levine and Fellows (22) compared the toxicity of acetic acid to that of lactic and hydrochloric acids on test organisms of food spoilage, namely, *Aspergillus niger* and different strains of bacteria. They concluded that the undissociated acetic acid molecule was toxic to microorganisms. The organisms, *Aspergillus niger*, *Salmonella aertrycke* and *Saccharomyces cerevisiae* were inhibited or destroyed at a higher pH value with acetic acid than with lactic or hydrochloric acids. Their results are recorded in table 2.

Bandelin (5) studied the effect of pH on the inhibiting efficiency of propionic acid on various moulds by visually comparing growth in innoculated Agar slants, which were stored at 30.0^{o}C for 14 days (table 3). The minimum concentration to inhibit mould growth at pH 3.0 and pH 5.0 did not change drastically. This indicates that the propionic acid is effective at both pH levels. However, the effect at pH 7.0 and pH 9.0 was nearly negligible at the maximum levels tested. These results also show, like acetic acid, that the undissociated molecule of propionic acid is responsible for toxicity.

TABLE 2: THE INHIBITING AND LETHAL ACETIC ACID CONCENTRATIONS FOR MICROORGANISMS (22).

Organism	inhibiting		lethal	
	ph*	acidity(%)	pH*	acidity(%)
Salmonella aertrycke	4.9	0.04	4.5	0.09
Staphylococcus aureus	5.0	0.03	4.9	0.04
Phytomonas phaseoli	5.2	0.02	5.2	0.02
Bacillus cereus	4.9	0.04	4.9	0.04
Bacillus mesentericus	4.9	0.04	4.9	0.04
Saccharomyces cerevisiae	3.9	0.59	3.9	0.59
Aspergillus niger	4.1	0.27	3.9	0.59

* the pH at which no visible growth occurred yet the microorganism remained viable.

** the pH at which total destruction took place.

TABLE 3: MINIMUM CONCENTRATION OF PROPIONIC ACID REQUIRED TO INHIBIT MOULD GROWTH (PERCENT IN MEDIA) (5).

pH	Moulds			
	Chaetomium globosum	*Alternaria solani*	*Penicillium citrinum*	*Aspergillus niger*
3.0	0.04	0.04	0.04	0.08
5.0	0.04	0.06	0.08	0.08
7.0	0.15	+	+	0.20
9.0	+	+	+	+

\+ indicates that mould growth occurred at 0.2% level, which was the maximum concentration of propionic acid used.

2.6.2 In-situ preservation.

Studies (32,37,38) have been carried out on the effect of reduced levels of chemical preservative on the keeping quality of grain. In all reports the high moisture grain was treated by spraying a preservative on the grain and placing the treated high moisture grain into storage at ambient conditions. The results showed that grains with 19.0 to 28.0% moisture content, which were treated with propionic and propionic-acetic acid mixtures at levels ranging from 50 to 100% of the recommended manufacturer's levels, kept well up to nine and one half months. In all instances, bacteria, yeasts and moulds were nearly eliminated. Mould development occurred in several samples and consisted almost entirely of *Aspergillus candidus*. This indicates that moulds, which may be resistant to propionic-acetic acid mixtures, may initiate spoilage in damp grain, especially grain treated with acid at marginal levels. Other investigators (9,23,33,39) have also arrived at the same conclusion, namely that reduced levels of preservative prevent mould from developing.

2.6.3 Nutritive value of chemically treated high moisture grain.

Feed trials conducted over a seven year period (1963-1969) at the Montana Agricultural Experimentation Station indicated that steers fed high moisture barley gained an average of 0.13 pounds per day more than steers fed dry barley (16). The feed trials also showed that there appeared to be an increase in the feed conversion efficiency in animals fed treated high moisture grain.

Jones (20) found that heifers fed corn treated with propionic acid had an 11% improvement in feed conversion. Young et al (44) concluded that the addition of propionic acid to high moisture corn gave equal or improved feed conversion performances in swine.

In 1970, at Olds, Alberta, a feed trial indicated that, on a comparable basis, chemically preserved high moisture barley was of greater value as a feedstuff for fattening steers than was dry barley (24).

Since propionic and acetic acids are naturally occurring acids in rumen fermentation (table 4) there is less than a 6.0% increase in propionate and acetate in the rumen when cattle consume 20 to 25 pounds of chemically treated high moisture grain. The results in table 4 are expressed in moles, and the equivalent gram values are presented in brackets.

TABLE 4: DAILY ACETATE AND PROPIONATE PRODUCTION IN THE RUMEN OF CATTLE (17).

Ration	Daily Production - moles (grams) Acetate	Propionate
Hay	14.4 (864.0)	5.05 (373.7)
Grain - hay	25.0 (1500.0)	8.95 (662.3)
Pasture	9.6 (576.0)	3.72 (275.3)

Hence, the feeding of chemically treated high moisture grain to animals would appear to have no adverse effects on the performance but, on the contrary, may have an additional energy value to the animal equivalent to that of the acid itself. Again, since the acids used in the treatments are naturally occurring organic compounds, they should present no ecological problems.

3. METHODS

3.1 Experimental procedure.

The detailed discussions of the methods used in this investigation, and the design and operation of the experiment are presented in the subsequent sections of this chapter.

The grain used was registered seed barley (*Hordeum olli*). For each of the chemical application rates, preweighed portions of the barley containing approximately 13.0% moisture were conditioned to the desired moisture content to yield a final sample weight of 1500 grams.

The individual 1500 gram quantities of conditioned barley were treated with marginal rates of Chemstor (table 7). Each 1500 gram sample was subdivided into ten 130 gram subsamples (referred to as 'units' hereafter) and placed in 250 milliliter Erlenmeyer flasks used as storage containers. The flasks were then stored either at 12.5°C or 28.0°C and connected to their respective humidity trains. The units were identified with respect to the temperature conditions imposed on each moisture - chemical combination (table 8). The remaining 200 grams of chemically treated barley were used for the initial moisture and acidity determinations.

Different temperature conditions were imposed on the units at 10-day and 20-day intervals when the units were removed from one temperature and placed into another.

Figure 5 shows a schematic diagram for the statistical design of the investigation.

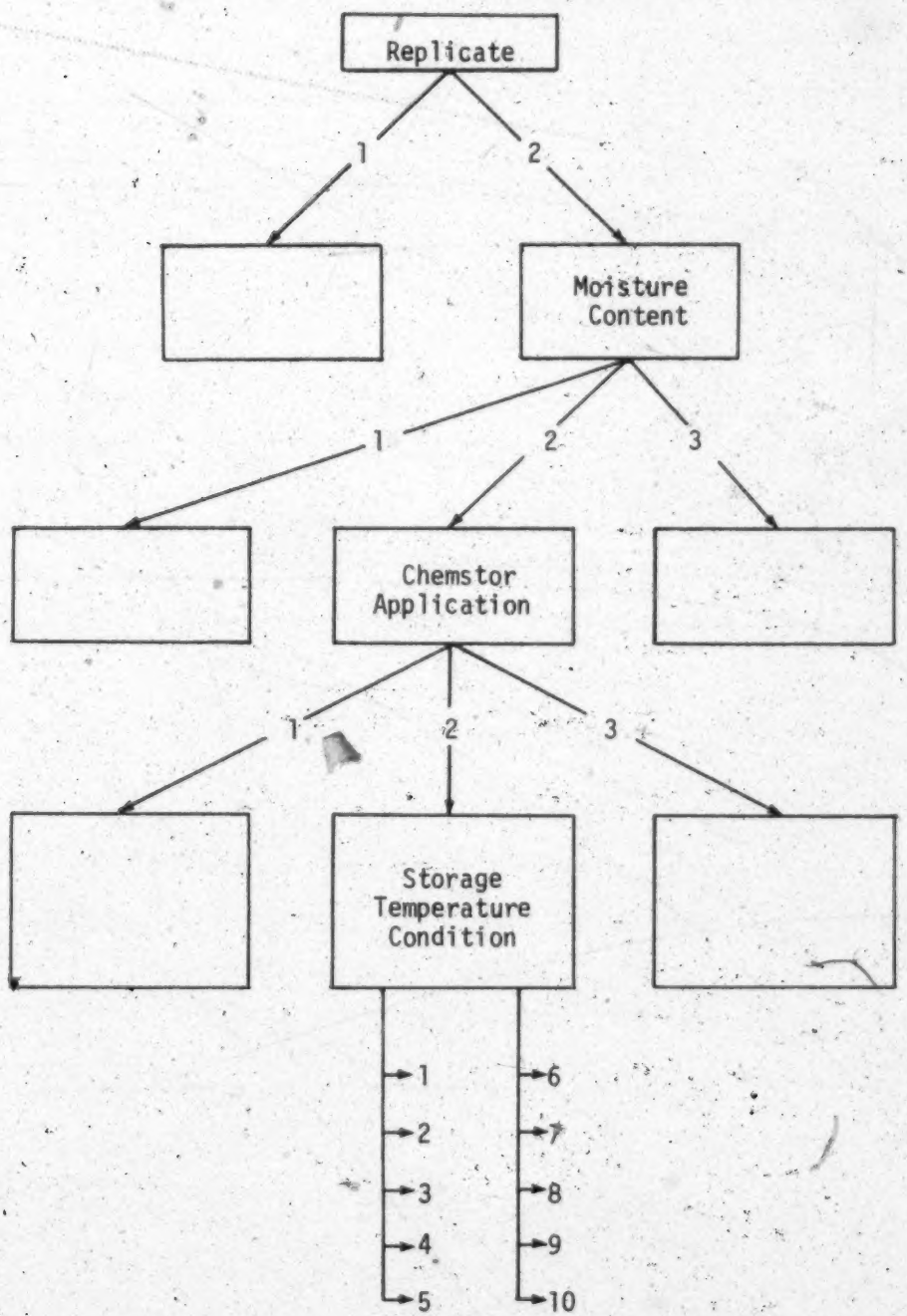

Figure 5: Schematic diagram of the statistical design.

3.2 Apparatus assembly.

Figure 6 shows a schematic diagram of the apparatus assembly for each temperature condition used in this experiment. Starting from the inlet laboratory air valve, there are seven essential elements to this train.

1. The inlet air was passed through a pressure regulator to reduce the air pressure from approximately eighty to five pounds per square inch. The air was passed through conditioning columns, consisting of four 9 inch x 3/4 inch O.D. glass columns closed at each end with one-hole rubber stoppers, to remove impurities, moisture and CO_2. The first two columns contained tightly packed glass wool to remove contaminates; the third column contained calcium chloride to remove any moisture present in the air; and the final column contained ascarite to absorb CO_2. Ascarite, which is a compound of asbestos and sodium hydroxide, changes color from light brown to white when spent. The ascarite's hygroscopicity necessitated the removal of water with calcium chloride prior to the removal of CO_2.

   The efficiency of the conditioning system appeared to be adequate as confirmed by the results of the experiment. For example, the conditioned air was checked periodically for the presence of CO_2 and none was detected by the method used for CO_2 determinations. Also, many samples produced little or no microbial growth on agar plates, suggesting that external contaminates were effectively removed.

2. The conditioned air then was saturated with water vapor by passing the air through sterilized distilled water contained

in a 750 milliliter Erlenmeyer flask. The air stream was broken up into bubbles by means of an air dispersion filter similar to the type used in fish tanks.

3. A three-way valve regulated the water-saturated air to pass through one of three moisture conditioning trains.

4. A moisture conditioning train consisted of two 750 milliliter Erlenmeyer flasks each containing a saturated salt solution that provided a relative humidity at a known temperature (table 5) which corresponded to the relative humidity in hygroscopic equilibrium with the treatments used in this experiment (table 6). The saturated air stream was dispersed through the saturated salt solution where a portion of the water vapor was absorbed by the salt solution yielding an air effluent corresponding to the desired relative humidity (42).

5. A two-way valve regulated the moisture-conditioned air to pass through either replicate one or replicate two of the samples of known moisture content.

6. A manifold distributed conditioned air to each flask. The distributing manifold was made of 3/4 inch O.D. hard copper tubing having fffteen 3/16 inch drilled holes with two inch pieces of soft copper tubing welded into each hole.

7. The storage containers (figure 7) consisted of 250 milliliter Erlenmeyer flasks closed with two-hole rubber stoppers, each fitted with an outlet-tube and an inlet-tube almost reaching to the bottom of the container. The flasks, when filled

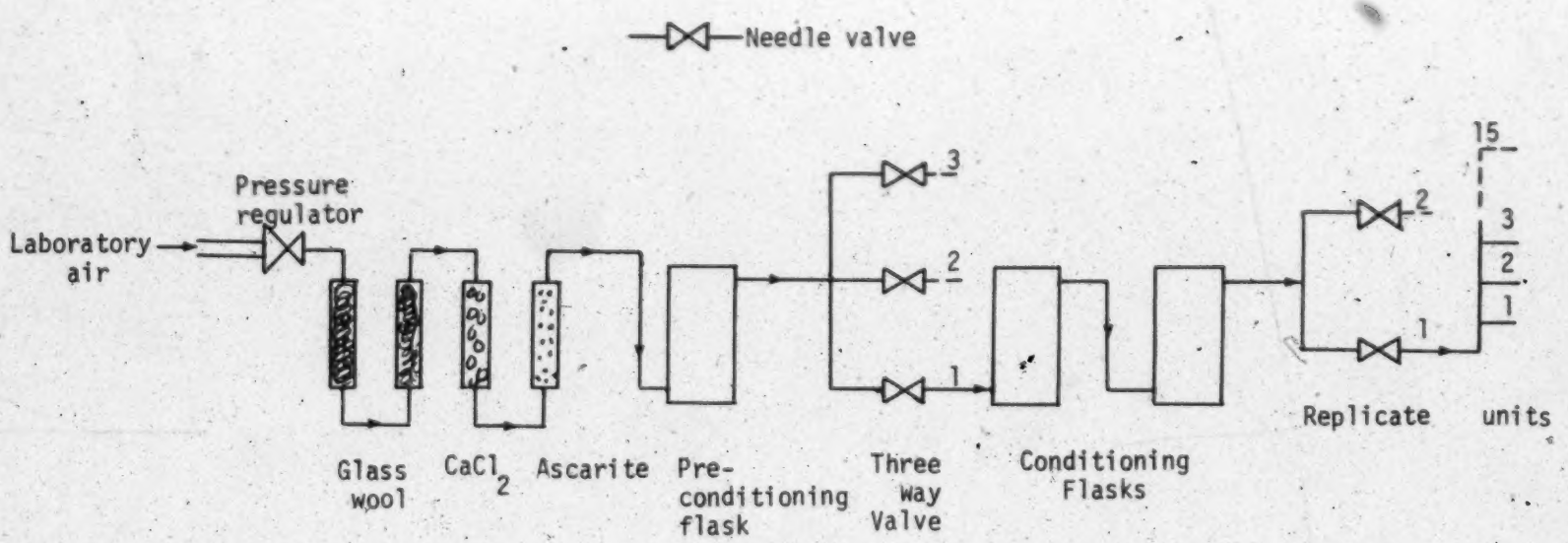

Figure 6: Schematic diagram of the humidifying train.

with treated grain, were attached to the distributing manifold corresponding to the desired relative humidity. A U-tube, attached to the outlet-tube of the flask and containing potassium hydroxide, entrapped the CO_2 produced by the grain samples.

TABLE 5: SATURATED SALT SOLUTIONS USED FOR RELATIVE HUMIDITY CONTROL FOR MOISTURE CONTENT-TEMPERATURE COMBINATIONS (42).

Temperature ^oC	Moisture content, percent 18.0	21.5	25.0
12.5	KCl		KNO_3
28.0	KNa Tartarate	KH_2PO_4	Water
37.0	$NH_4H_2PO_4$	K_2SO_4	Water

Table 6: BARLEY EQUILIBRIUM RELATIVE HUMIDITIES, PERCENT*.

Temperature ^oC	Moisture content, percent 18.0	21.5	25.0
12.5	83.0	90.5	95.4
28.0	87.5	94.4	98.5
37.0	90.0	96.1	100.0

* summarized from figure 1.

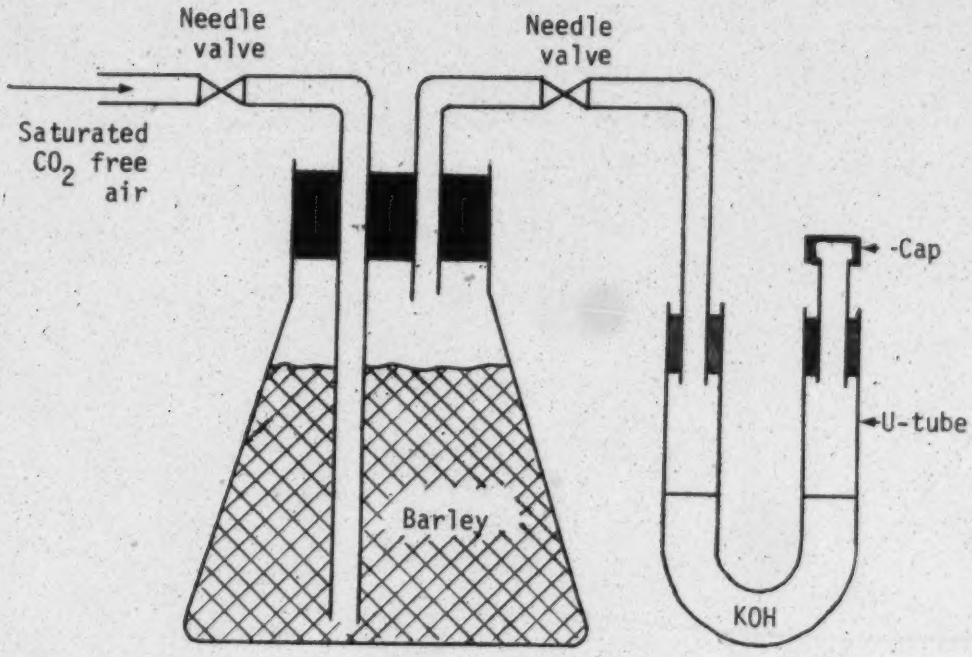

Figure 7: Schematic diagram of an assembled unit.

3.3 Moisture conditioning.

Grain is 'hygroscopic' meaning that absorption or adsorption of moisture will occur until the grain is in equilibrium with the surrounding air. Two methods commonly used to condition grain to the required moisture content are:

1. the direct application of the water to the grain bulk, and
2. the passage of humidified air, corresponding to the required moisture content, through the grain bulk.

Hustrulid (18), using small amounts of wheat, found that the characteristics of naturally moist and remoistened kernels were not significantly different.

In this experiment, the water was applied directly to the grain bulk. The following formula was used to calculate the weight of barley required to obtain a final weight of 1500 grams at the desired moisture content for each of the three moisture contents used.

$$W_1 = \frac{100 - M.C._2}{100 - M.C._1} W_2$$

where W_1 = weight of unconditioned grain (grams),

W_2 = weight of conditioned grain (grams),

$M.C._1$ = initial moisture content of the unconditioned grain, and

$M.C._2$ = final moisture content of the conditioned grain.

The preweighed unconditioned barley was placed into a double-walled polyethylene bag. Since the specific gravity of water is 'one', the difference between 1500 grams and the initial weight of barley equalled the amount of water necessary to increase the moisture content of the grain sample to the desired level, and also obtain a final

weight of 1500 grams. The water was sprayed on the kernels in a two step procedure where one half of the required water was added initially, and the remainder 24 hours later. During the conditioning, the bags were stored at 4.0°C for four days during which time the bags were frequently agitated to provide equal distribution of moisture between the kernels.

3.4 Moisture determination.

Moisture content determinations were carried out in accordance with the standard air-oven method (12). The standard method was modified to minimize the amount of grain required for each moisture determination. The extent of modification was the use of three replicates of 40 to 50 grams of whole kernels of the moisture tempered grain for the initial moisture content. The initial moisture content of the grain sample consisted of the moistened grain plus the applied preservative. The possibility of the actual preservative increasing the moisture content was assumed to be negligible.

For final moisture determinations, one replicate of 40 to 50 grams was used from each unit as each unit sample initially weighed 130 grams from which approximately 35 grams were required for microbial analysis and a further 45 grams for acidity determinations. All samples were placed in a forced air oven at 130.0°C for 20 hours. The loss in weight was expressed as a percentage of the total sample weight to give the grain moisture content on a wet basis.

3.5 Application of the chemical preservative.

Table 7 shows the recommended and experimental application rates of 'CHEMSTOR'. The specific gravity of Chemstor was calculated in relative proportions of the acetic and propionic acid content and was found to be 1.035. For simplicity, the specific gravity was assumed

to be 1.0. Calculation of the amount of Chemstor required for the treatment of 18% moisture grain at 25% of the recommended value yielded 2.4 milliliters, an amount considered too small to distribute evenly on 1500 grams of grain wihtout sophisticated application equipment. Since acetic acid and propionic acid are readily soluble in water, appropriate water - preservative mixtures were prepared. These volumes consisted of three times the calculated application amount plus sufficient distilled water to give a total of 45 milliliters of solution. Fifteen milliliters of this solution were sprayed on the 1500 grams of grain. The individual 1500 gram portions of the moisture-conditioned grain were placed in a stainless-steel wire-mesh drum which was rotated at 50 r.p.m. for 30 seconds while the preservative was sprayed on the grain with a fine spray nozzle.

In a preliminary study, it was found that, when using water colored with crystal violet stain, the application of 15 milliliters of solution to a drum containing 1500 grams of grain rotating at 50 r.p.m. for 30 seconds resulted in all kernels receiving some solution. Grain samples were examined for crystal violet specks.

TABLE 7: EXPERIMENTAL CHEMSTOR APPLICATION RATES, PERCENT BY WEIGHT.

Moisture Content, percent	Recommended Rate (7)	Experimental rate percent of recommended rate		
		25	50	75
18.0	0.80	0.20	0.40	0.60
21.5	0.98	0.25	0.49	0.74
25.0	1.20	0.30	0.60	0.90

3.6 Acidity determination.

Propionic, acetic and formic acids contain the carboxyl group

$$-\overset{\overset{O}{\parallel}}{C}-OH$$

and, therefore, are referred to as carboxylic acids. The quantity of carboxylic acid present in a solution can be determined by titrating the solution with a base. The carboxylic acids combine with bases to form salts which hydrolyze in an aqueous solution. For example, the titration of acetic acid with sodium hydroxide yields a salt (CH_3COONa) as in the following equation:

$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O$$

Neutralization of the acid and base occurs at the 'equivalence point'; the neutralization equivalent may be defined as the number of grams of acid required to neutralize one gram equivalent of alkali (10). The pH of the solution at which the equivalence point occurs can be determined with phenolphthalein, an indicator which turns pink at pH 8.3.

The experimental procedure for determining the acidity content of the grain was similar to that used by the manufacturer of Chemstor, and is outlined as follows:

1. a sample of whole kernels weighing 15 grams was placed in an osterizer containing approximately 100 milliliters of distilled water,
2. the mixture was osterized at full speed for five minutes,
3. the mixture then was placed in a beaker and the osterizer was washed with distilled water to ensure complete acid removal, and

4. the beaker was placed on a magnetic stirrer and the contents were titrated with a standardized sodium hydroxide solution to pH 8.3. A Beckman "Zeromatic II" Model 96A pH meter was used to determine the pH endpoint.

3.6.1 Acidity calculations.

The following formula was used to determine the Chemstor acidity of the grain:

$$\text{percent Chemstor acidity} = \frac{N_{NaOH} \times \text{ml NaOH} \times \text{equivalent weight}}{1000 \times \text{sample weight (grams)}} \times 100$$

where: ml. NaOH = the amount of standardized NaOH added to the solution and corrected for the background acidity of the grain, and

N_{NaOH} = the normality of the NaOH solution.

The equivalent weight of Chemstor was determined in accordance with the percentage of acetic and propionic acid in the preservative. Acetic and propionic acid have molecular weights, respectively, of 60 and 74. The equivalent weight of Chemstor is 60 x 0.60 (60% acetic acid) plus 74 x 0.40 (40% propionic acid) equalling 65.6.

3.6.2 Standardization of sodium hydroxide.

The sodium hydroxide solution was standardized against hydrochloric acid of a known concentration.

3.6.3 Frequency of grain acidity determinations.

The background acidity of the untreated grain was determined for each moisture level. The total acidity for each

moisture Chemstor application rate combination was determined from the average of three 15 gram samples of grain randomly withdrawn from the 1500 grams of chemically treated grain. The percent Chemstor acidity was obtained by subtracting the background acidity from the total acidity.

The final Chemstor acidities were determined whenever a unit was withdrawn from the experiment. Normally, the average of three determinations was used to determine the final total acidity content for each unit. However, when two successive determinations from the same unit were duplicated, a third determination was omitted, and the final total acidity was based on the two determinations.

The final Chemstor acidity was obtained by subtracting the background acidity (recorded as the initial background acidity of the untreated grain) from the final acidity.

3.7 Carbon dioxide determinations.

Carbon dioxide is fixed as potassium carbonate (K_2CO_3) when it is absorbed by potassium hydroxide (KOH). The amount of CO_2 fixed as K_2CO_3 can be determined by titrating the sample with hydrochloric acid (HCl) in which the following reactions occur:

$$2KOH + CO_2 \longrightarrow K_2CO_3 + H_2O \quad (1)$$

$$K_2CO_3 + HCl \longrightarrow KHCO_3 + KCl \quad (2)$$

$$KHCO_3 + HCl \longrightarrow H_2CO_3 + KCl \quad (3)$$

The reactions in equations (1), (2) and (3) occur respectively at points A, B and C on the carbonate-hydrochloric acid titration curve (figure 8).

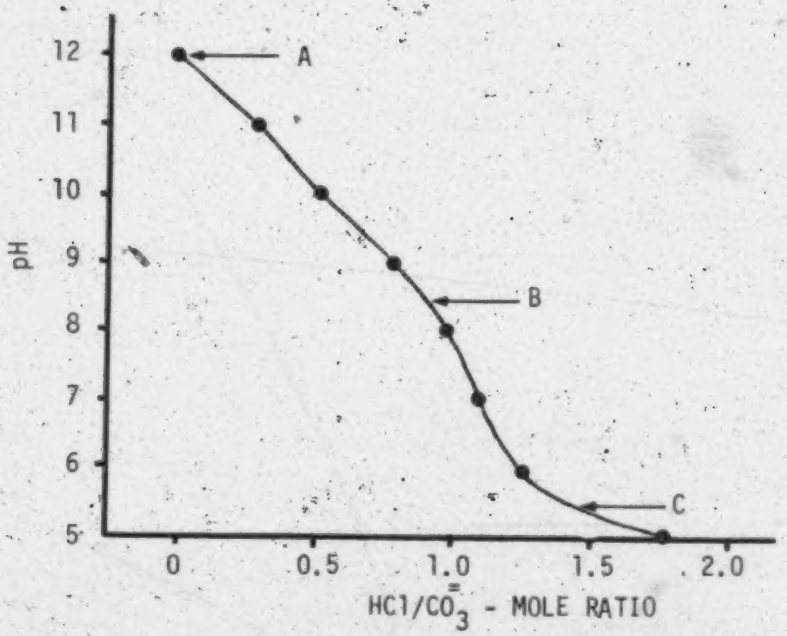

Figure 8: Curve for the titration of carbonate with hydrochloric acid (21).

Point A: The solution contains K_2CO_3, water and any KOH that was not fixed by the CO_2.

Point B: The addition of HCl to the solution causes the carbonate to change to a bicarbonate ($KHCO_3$). At this equivalence point of pH 8.3, all the hydroxide and half of the carbonate have been neutralized. At this point, the solution contains only potassium bicarbonate.

Point C: Subsequent addition of HCl results in the occurrence of carbonic acid (H_2CO_3). At this point, the pH is 5.5. The amount of standardized HCl required to lower the pH from 8.3 to 5.5 was used to calculate the amount of CO_2 present in a sample.

The following procedure was used to determine the amount of CO_2 produced in each unit:

1. the contents of the U-tube were poured into the beaker and the U-tube was washed with distilled water,
2. the solution was titrated with approximately 0.5 normal (N) HCl to an end-point of pH 8.3, using phenolphthalein as a color indicator which changes from pink to colorless. An exact concentration of HCl is not necessary since the existing KOH in solution is neutralized, and
3. standardized 0.1 N HCl was added to lower the pH from 8.3 to 5.5 using methyl-red as a color indicator which changes from yellow to red. A standard color blank was prepared for pH 5.5 and all samples were titrated to the standardized color.

The amount of added standardized 0.1 N HCl was used to calculate the CO_2 produced, using the following formula:

$$\text{mg } CO_2/100 \text{ grams dry weight/unit time} = \text{ml. HCl} \times N_{HCl} \times \text{correction factor} \times 44.$$

where: correction factor = total weight of high moisture barley corrected to 100 grams dry weight.

All the CO_2 production data was calculated on a 100 gram dry weight basis.

3.7.1 Frequency of carbon dioxide determinations.

Initially, carbon dioxide determinations were conducted every ten days. As the experiment progressed, it was noted that very little CO_2 could be detected. Therefore, the frequency of CO_2 determination was reduced to the following schedule. After 50 days,

CO_2 determinations were conducted at 70, 90, 100, 125 and 150 days. The results from the 70, 90 and 100-day determinations were averaged over ten day intervals; namely, 60 and 70 days for the 70-day determination and 80 and 90 days for the 90-day determination. The CO_2 determinations for 100, 125 and 150 days were recorded as determined.

3.7.2 Frequency of aeration.

The units were aerated every five days up to the 50-day CO_2 determination interval. Thereafter, the units were aerated every 10 days to the end of the experiment at 150 days.

3.8 Microbial analysis.

3.8.1 Frequency of analysis.

Each unit was analyzed for its microbial content at 50, 100 and 150 day intervals. If a unit exhibited microbial growth, whether it was signaled by a sudden increase in CO_2 production or from visual observation of mould growth, that unit was also analyzed.

3.8.2 Removal of grain samples.

Using 'aseptic' techniques, approximately 13 grams of grain were removed from each unit, placed in sterilized test tubes, sealed and stored at $4^{o}C$ until microbial determinations could be conducted. The following aseptic procedure was used to remove individual grain samples from each unit:

1. The rubber stopper was removed from the Erlenmeyer flask, and the mouth of the flask was flamed.
2. The spatula, which was used to remove the grain kernels, was placed in ethyl alcohol, withdrawn and flamed. Sufficient grain kernels were removed and placed in sterilized test tubes.

3. The mouth of the Erlenmeyer flask was reflamed, the rubber stopper replaced, and the flask replaced at the temperature of storage.

3.8.3 Preparation of dilutions.

Ten grams of barley were placed in a sterile dilution bottle containing 90 mls. of sterile distilled water yielding a 1:10 dilution. The dilution bottle then was placed on a horizontal shaker for 20 minutes at 276 revolutions per minute.

Subsequent dilutions of 1:100 and 1:1000 were prepared in sterile distilled water as follows, by pipetting with a sterile pipette:

Dilutions: 1:100 - 10 mls of 1:10 dilution + 90 mls water
1:1000 - 10 mls of 1:100 dilution + 90 mls water.

Each dilution bottle was shaked to obtain homogenity; 25 complete up-and-down movements, of about three feet.

3.8.4 Quantification of the microbial load.

Plating for bacteria and moulds consisted of using 0.1 and 1.0 mls. of the 1:10 dilution for units stored at 37.0°C or units treated with Chemstor levels C_2 and C_3. Appropriate higher dilutions were plated using 0.1 mls of suspension for units treated at Chemstor level C_1 and stored at 12.5°C or 28.0°C. Three replicates for each dilution used were plated.

3.8.4.1 Bacterial quantification.

Plate Count Agar was used to determine the number of bacteria present on the grain kernels. The plates were stored at 28.0°C for 48 hours. Plates containing between 30 and 300 colonies were counted. The average of three plates was used for the total

bacterial count, expressed as the number of viable bacteria per one gram of wet weight.

3.8.4.2 Mould quantification.

Phytone Yeast Extract Agar containing 0.01% weight per weight actidione (a bacterial inhibitor) was used to culture the moulds. The plates were stored at 28.0°C for 72 hours and developed colonies were counted. The plates were replaced at 28.0°C and stored for sufficient time for further development of the mould colonies for identification.

3.8.5 Mould identification.

The moulds were characterized according to physical morphology, that is, color, shape and size. Isolates were prepared from groups of moulds having the same characteristics and then sent to a mycologist for identification.

3.9 Removal of a unit.

Units were removed from the experimental conditions whenever a sudden rise in CO_2 production signalled mould development or whenever moulds were visually observed. The length of storage for each unit removed was arbitrarily chosen by the author. Characteristics such as the amount of CO_2 produced, extent of moulding and total moulds and bacteria present influenced the recorded storage time.

3.10 Experimental design.

The experiment was of a factorial design consisting of two replicates, three moisture contents, three Chemstor application rates within each moisture content and ten temperature conditions.

4. ANALYSIS OF DATA

4.1 Methods of data analysis.

The methods of analyzing the data consisted of statistical procedures involving analysis of variance and Duncan's New Multiple Range Test (36).

4.1.1 Independent variables.

The source of variation, their codes and subscripts used in the analysis are given in table 8.

4.1.2 Type of data.

For the analysis of variance, periodic CO_2 production, final Chemstor acidity values, final pH, final moisture content, and length of storage data were considered. The actual values for the parameters measured are given in Appendices A and B.

4.2 Analysis of data - analysis of variance.

The analysis of variance of the data was carried out on the basis of a split plot design with moisture content (M) and Chemstor application rates within moisture content (C/M) in the whole plot and the temperature conditions (T) in the subplot.

The calculations involved in the analysis of variance were made using a modified version of a University of Alberta Computing Centre Library Program (41). The extent of the modification was the printing of the F-values and the probabilities of variance ratios on the computer output sheets.

The model for the analysis of variance was:

$$Y_{ijklm} = u + R_i + M_j + C_{k(j)} + T_l + TC_{lk(j)} + TM_{jl} + RM_{ij} + RC_{ik(j)} + TR_{il} + TRM_{ijl} + TRC_{ilk(j)}$$

where Y_{ijklm} = the measurement of the i^{th} replicate of the j^{th} moisture content of the $k(j)^{th}$ chemical application rate within moisture of the l^{th} temperature treatment.

$i = 1,2$

$j = 1,2,3$

$k = 1,2,3$

$l = 1,2, \ldots, 10$

The expected mean squares (table 9) were computed assuming replicates as random sources of variation, with moisture content and Chemstor application rate within moisture and temperature as fixed sources of variation (15). The interactions with replicates were combined to form the error terms. The whole plot error (error 1) consisted of the interaction of replicate with moisture (RM) and Chemstor application rate within moisture (RC/M). The sub-plot error (error 2) consisted of the interaction of replicate with temperature (RT), temperature by moisture content (RTM) and temperature by Chemstor application rate within moisture (RTC/M).

TABLE 8: LIST OF VARIABLES, CODES, SUBSCRIPTS AND THE LEVEL OF EACH USED.

Variable	Code	Subscript	Level
Replicate	R	1	
		2	
Moisture Content	M	1	18.0% moisture grain.
		2	21.5% moisture grain.
		3	25.0% moisture grain.
Chemstor Application Rate	C	1	25% of the recommended application rate.
		2	50% of the recommended application rate.
		3	75% of the recommended application rate.
Temperature Conditions	T	1	initial temperature - 12.5°C; 28.0°C after 10 days of storage.
		2	initial temperature - 12.5°C; 37.0°C after 10 days of storage.
		3	initial temperature - 12.5°C; 28.0°C after 20 days of storage.
		4	initial temperature - 12.5°C; 37.0°C after 20 days of storage.
		5	maintained at the initial temperature of 12.5°C.
		6	initial temperature - 28.0°C; 12.5°C after 10 days of storage.
		7	initial temperature - 28.0°C; 37.0°C after 10 days of storage.
		8	initial temperature - 28.0°C; 12.5°C after 20 days of storage.
		9	initial temperature - 28.0°C; 37.0°C after 20 days of storage.
		10	maintained at the initial temperature of 28.0°C.

TABLE 9: TABLE OF EXPECTED ERROR MEAN SQUARES (E.M.S.)

Source of variation	Degrees of freedom	E.M.S.
R_i	1	$\sigma^2_\varepsilon + 90\,\sigma^2_R$
M_j	2	$\sigma^2_\varepsilon + 30\,\sigma^2_{RM} + 60\,\sigma^2_M$
$C_{k(j)}$	4	$\sigma^2_\varepsilon + 10\,\sigma^2_{RC} + 2\,\sigma^2_C$
RM_{ij}	2	$\sigma^2_\varepsilon + 30\,\sigma^2_{RM}$
$RC_{ik(j)}$	4	$\sigma^2_\varepsilon + 10\,\sigma^2_{RC}$
T_l	9	$\sigma^2_\varepsilon + 9\,\sigma^2_{TR} + 18\,\sigma^2_T$
TR_{il}	9	$\sigma^2_\varepsilon + 9\,\sigma^2_{TR}$
TM_{jl}	18	$\sigma^2_\varepsilon + 3\,\sigma^2_{TRM} + 6\,\sigma^2_{TM}$
$TC_{lk(j)}$	36	$\sigma^2_\varepsilon + \sigma^2_{TRC} + 2\,\sigma^2_{TC}$
TRM_{ijl}	18	$\sigma^2_\varepsilon + 3\,\sigma^2_{TRM}$
$TRC_{ilk(j)}$	36	$\sigma^2_\varepsilon + \sigma^2_{TRC}$
$error_{m(ilk(j))}$	40	σ^2_ε
	179	

4.2.1 Analysis of variance (Modified).

The analysis of variance model in section 4.2 was used for the analysis of variance on the 10,20,30,40 and 50-day CO_2 production, final Chemstor acidity, final pH, final moisture content and length of storage data. For the 60-day and subsequent CO_2 production data, the analysis of variance model was modified to accommodate the removal of a unit from the experiment, because of microbial development, thus

resulting in a non-orthogonal statistical design. Orthogonality for the analysis of variance was maintained by the following techniques:

1. The CO_2 production data from the units containing high moisture barley treated with the lowest Chemstor application rate within each moisture content were not included in the modified analysis of variance procedures, and
2. the average CO_2 production from the remaining units maintained under the same experimental conditions was substituted when a unit containing high moisture barley, treated with higher Chemstor application rates within each moisture content, was removed due to microbial development.

The modified E.M.S. used in the analysis of variance on the 60-day and subsequent CO_2 determination periods are given in table 10.

4.3 Duncan's New Multiple Range Test

Using appropriate procedures as detailed by Steel and Torrie (36), Duncan's New Multiple Range Test, or Duncan's Test, was carried out on the means of factors and/or factor interactions that indicated significant differences when tested in the analysis of variance.

TABLE 10: TABLE OF EXPECTED ERROR MEAN SQUARES (MODIFIED).

Source of Variation	Degrees of freedom	E.M.S.
R_i	1	$\sigma_\varepsilon^2 + 60\,\sigma_R^2$
M_j	2	$\sigma_\varepsilon^2 + 20\,\sigma_{RM}^2 + 40\,\sigma_M^2$
$C_{k(j)}$	2	$\sigma_\varepsilon^2 + 2\,\sigma_C^2 + 10\,\sigma_{RC}^2$
RM_{ij}	2	$\sigma_\varepsilon^2 + 30\,\sigma_{RM}^2$
$RC_{ik(j)}$	2	$\sigma_\varepsilon^2 + 10\,\sigma_{RC}^2$
T_l	9	$\sigma_\varepsilon^2 + 6\,\sigma_{TR}^2 + 12\,\sigma_T^2$
TR_{il}	9	$\sigma_\varepsilon^2 + 6\,\sigma_{TR}^2$
TM_{jl}	18	$\sigma_\varepsilon^2 + 2\,\sigma_{TRM}^2 + 4\,\sigma_{TM}^2$
$TC_{lk(j)}$	18	$\sigma_\varepsilon^2 + \sigma_{TRC}^2 + 3\,\sigma_{TC}^2$
TRM_{ijl}	9	$\sigma_\varepsilon^2 + 2\,\sigma_{TRM}^2$
$TRC_{ilk(j)}$	18	$\sigma_\varepsilon^2 + \sigma_{TRC}^2$
$error_{m(ilk(j))}$	29	σ_ε^2
Total	119	

5. RESULTS

The chosen grain moisture contents were within the range that high moisture grain may be harvested, while the Chemstor application rates were considered to be sufficiently low to allow microflora to develop and proliferate. The chosen storage temperatures simulated conditions that may occur during harvesting, caused by ambient temperatures and solar radiation, and during storage resulting from microbial and grain respiration and ambient temperature fluctuations. However, there were a number of factors which may deviate from practice. In stored grain, forced aeration does not occur; the gases diffuse through the grain bulk instead. In this experiment, intermittent aeration of the units was necessary as the CO_2 produced was used as an index of microbial development.

5.1 Initial conditions.

The initial experimental moisture contents, pH and Chemstor application rates are summarized in table 11. The overall moisture contents for moisture levels M_1(18.7%) and M_2(22.1%) were respectively 0.7% and 0.6% higher due to a malfunction of the spray equipment during the conditioning procedure of replicate one. No overall moisture content differences existed in moisture level M_3(25.0%).

The actual overall Chemstor application rates obtained were 29, 41 and 51% as compared to the theoretical values of 25, 50 and 75% of the recommended application rates. The vaporization of the Chemstor into the air during spraying of the barley samples may account for the rates being lower than those originally considered.

TABLE 11: INITIAL MOISTURE CONTENT, pH AND CHEMSTOR ACIDITY OF THE BARLEY.

	Replicate	Moisture level 1			2			3		
		Level of Chemstor Application 1	2	3	1	2	3	1	2	3
Moisture content, percent	1	19.4	19.7	19.1	22.7	22.6	22.4	24.7	24.7	24.6
	2	18.0	18.0	17.9	21.8	21.2	21.5	25.2	25.1	25.5
Overall average			18.7			22.6			25.0	
pH	1	5.50	5.20	5.00	5.30	5.10	5.00	5.10	5.00	4.90
	2	5.30	5.10	5.00	5.20	5.00	4.90	5.10	4.90	4.80
Average		5.10	5.15	5.00	5.25	5.05	4.95	5.10	4.95	4.85
Chemstor acidity*	1	0.19	0.29	0.42	0.25	0.36	0.44	0.37	0.50	0.57
	2	0.25	0.34	0.42	0.30	0.43	0.49	0.37	0.52	0.66
Average		0.28	0.39	0.52	0.36	0.51	0.60	0.49	0.68	0.82
Percent of recommended Chemstor		28	40	53	28	40	48	31	43	51

* Expressed as percent per 100 grams dry matter.

5.2 Carbon dioxide production.

The overall mean values for the different variables are presented to show the trends. Table 12 shows the overall mean CO_2 production for each temperature condition. A total of 18 units was used to obtain the average value for each temperature condition for 10 to 50 days. The average of 12 units was used to obtain the values for 60 to 150 days. Generally, after 30 days, the mean CO_2 production for each temperature condition decreased until a stabilized respiration rate was achieved.

TABLE 12: THE MEAN CO_2 PRODUCTION FOR THE TEMPERATURE CONDITIONS, mg CO_2/100 grams/unit time*.

Days	Temperature condition**									
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
10	62.7	66.5	61.8	63.9	66.6	173.7	159.2	178.3	172.5	178.5
20	127.5	161.7	31.1	33.2	34.5	26.2	86.2	78.3	78.6	82.1
30	62.8	15.4	101.7	117.6	19.9	13.8	12.3	11.8	45.7	34.1
40	33.4	11.8	55.6	11.8	12.9	8.5	10.2	6.3	11.4	22.3
50	29.0	10.5	45.1	9.9	11.4	7.5	11.4	6.2	12.9	26.7
60	4.2	9.0	5.7	9.7	6.5	3.6	8.7	2.5	9.0	19.2
70	3.9	9.2	3.5	9.9	6.1	3.7	10.2	2.3	8.2	3.9
80	4.3	9.3	3.3	10.1	6.1	3.9	10.2	2.2	10.2	4.1
90	5.8	8.9	3.9	9.6	5.9	3.9	8.9	2.3	10.9	4.3
100	5.7	8.8	4.0	9.9	6.1	4.2	9.0	2.4	11.3	7.4
125	21.9	20.8	11.4	21.7	10.4	6.7	20.8	4.9	19.5	25.6
150	7.2	15.9	14.6	15.8	10.4	5.7	17.2	3.1	14.4	7.4

* From 10 - 50 days, the average of 18 units was used for the mean CO_2 production, whereas from 60 - 150 days, the average of 12 units was used for the mean CO_2 production.

** The description of the temperature conditions are given in table 8.

Table 13 shows the overall mean CO_2 production rates for each moisture Chemstor application rate combination. With the exception of the treated 18.7% moisture grain, the mean CO_2 produced from each moisture content decreased with an increase in Chemstor application rate.

TABLE 13: THE RESPIRATION OF HIGH MOISTURE GRAIN AT VARIOUS CHEMSTOR ACIDITIES, mg CO_2/100 grams/10 days.

Initial Moisture Content, percent	Initial Chemstor Acidity, percent per 100 grams dry matter	Storage days 10	20	30	40	50
	0.28	80	101	85	37	35
18.7	0.39	88	76	51	16	9
	0.52	67	52	39	13	8
	0.36	201	136	72	23	27
22.6	0.51	151	81	41	13	9
	0.60	95	49	29	10	9
	0.49	245	118	51	39	44
25.5	0.68	83	35	15	8	7
	0.82	56	17	10	7	7

Figure 9 shows the overall mean CO_2 production for the marginal Chemstor application rates. The respiration of the high moisture barley treated with 41% and 51% of the recommended Chemstor application rate decreased to a mean of 6 mg. CO_2 per 10 days at 50 days of storage. A respiration rate of 32 mg. CO_2 per 10 days was recorded at 40 and 50 days of storage at 29% of the recommended Chemstor application rate.

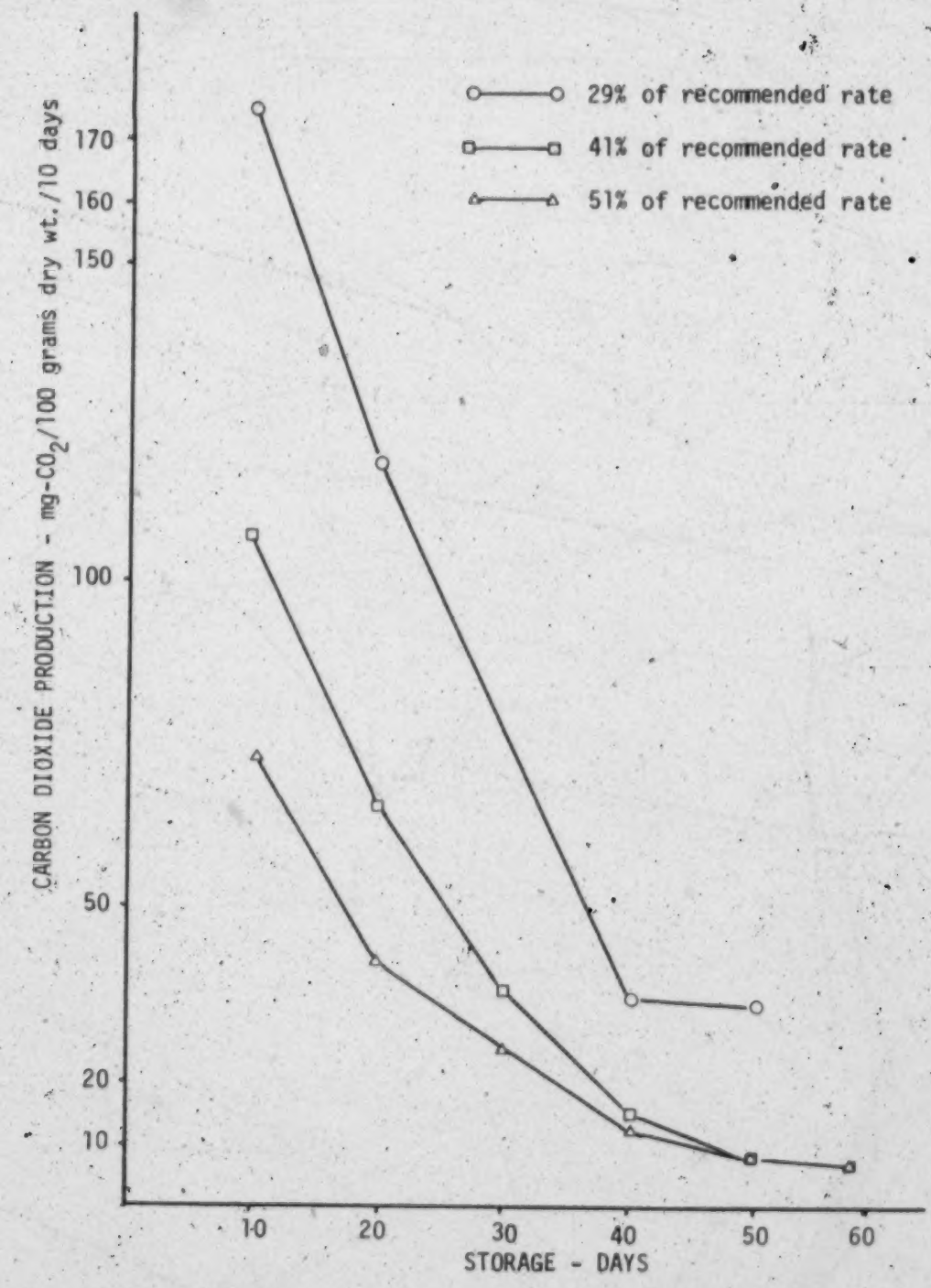

Figure 9: Influence of the overall mean marginal Chemstor application rates on respiration.

Figure 10 shows the mean respiration rate due to moisture content. Normally, increases in CO_2 production are associated with increases in moisture content (3). However, the amount of Chemstor applied affected the CO_2 production rate. At 10 and 20 days, the CO_2 produced from the 22.1% moisture grain was greater than that produced from the 18.7% moisture grain, but the CO_2 produced from the 25% moisture grain was not greater than that produced from the 22.1% moisture grain. At 30 days, increasing moisture contents were associated with decreasing CO_2 production. At 40 and 50 days, the overall mean CO_2 production ranged by 7 mg. and 3 mg. respectively.

5.2.1 Analysis of variance - carbon dioxide.

Tables 14 and 15 respectively give the analysis of variance for the CO_2 determination intervals for three levels of C/M (C_1, C_2, and C_3) and two levels of C/M (C_2 and C_3). The computed F-values indicated that the main effects due to C/M, moisture content and temperature were significant.

The C/M was highly significant at 10, 20, 30, 40 and 50 days. The effect of moisture was highly significant at 10, 20 and 30 days. The effect of temperature was highly significant for all CO_2 determination intervals irrespective of the number of levels of Chemstor application rates used in the analysis of variance.

At 60 days***, the analysis of variance was conducted using a high CO_2 production of 180 mg. for unit $R_2M_3C_2T_{10}$. As a result, one high CO_2 production value negated any significant effects that may have existed.

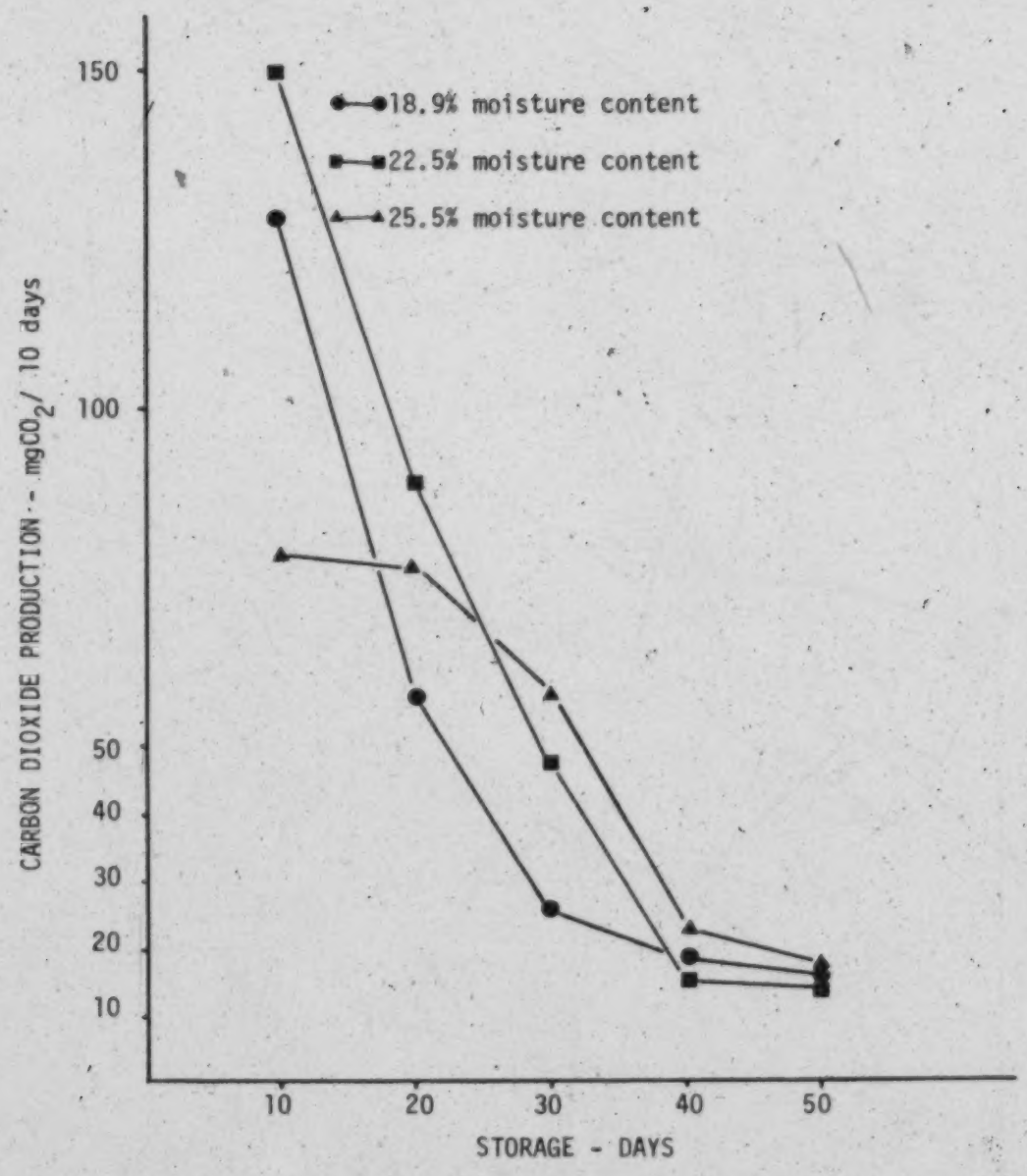

Figure 10: Graph illustrating the relationship between various Chemstor treated moisture conditions and respiration.

TABLE 14: ANALYSIS OF VARIANCE - CO_2 PRODUCTION.

Source of variation	Degrees of Freedom	10 days Mean Square	10 days F-value	20 days Mean Square	20 days F-value	30 days Mean Square	30 days F-value	40 days Mean Square	40 days F-value	50 days Mean Square	50 days F-value
R	1	43031.0	12.06**	10065.0	4.29	5563.3	4.23	1466.6	2.84	970.7	1.90
M	2	79911.0	22.40**	15822.0	6.75*	16701.0	12.71**	693.1	1.34	344.8	<1
C/M	6	89591.67	25.11**	36120.0	15.41**	10492.7	7.98**	3716.4	7.19**	5281.1	10.33**
Error	8	3567.6		2343.7		1314.5		516.8		511.0	
T	9	59015.0	95.34**	36298.0	122.80**	27120.0	98.92**	4205.2	29.04**	2812.5	21.06**
T x M	18	2110.0	3.41**	2572.3	9.31**	2834.2	10.34**	353.8	2.44**	153.3	1.01
T x C/M	54	1851.0	2.99**	1589.9	5.38**	1299.0	4.74**	548.1	3.78**	933.6	6.99**
Error	81	619.0		295.6		274.2		144.8		133.6	

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

TABLE 15: ANALYSIS OF VARIANCE (MODIFIED) - CO_2 PRODUCTION.

Source of Variation	60 days***		60 days		70 days		80 days	
	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
R	57.82	<1	74.42	7.56*	91.00	16.03*	131.88	45.41**
M	235.98	<1	36.25	3.68	20.49	3.61	32.89	11.32*
C/M	189.41	<1	12.20	1.24	21.86	3.79	16.89	5.81*
Error (1)	316.13		9.84		5.68		2.90	
T	269.30	<1	81.82	9.80**	109.86	18.10**	125.10	24.41**
T x M	275.64	1.02	32.45	3.89**	24.54	4.04**	31.36	6.12**
T x C/M	269.45	<1	4.01	<1	4.98	<1	4.39	<1
Error (2)	271.36		8.35		6.07		5.13	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Unit $R_2M_3C_2T_{10}$ = 180 mg CO_2.

TABLE 15: CONTINUED

Source of Variation	90 days		100 days		125 days		150 days	
	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
R	118.60	6.51	195.84	3.86	2371.8	2.44	173.04	1.53
M	94.97	5.21	108.21	2.13	1176.7	1.21	141.43	1.25
C/M	10.19	<1	36.73	<1	955.2	<1	35.43	<1
Error (1)	18.21		50.82		971.3		112.75	
T	102.59	8.98**	99.48	3.16**	628.6	1.36	303.49	3.90**
T x M	46.72	4.09**	73.32	2.33**	589.3	1.27	118.28	1.52
T x C/M	12.10	1.06	31.27	<1	409.89	<1	75.76	<1
Error (2)	11.42		31.53		463.9		77.86	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

5.3 Final Chemstor acidity values.

For the purpose of statistical analysis, the final Chemstor acidity values (Appendix B) were corrected to 100 grams dry weight basis. The final mean Chemstor acidity values due to temperature, moisture and C/M are shown in table 16. Where applicable, the initial Chemstor acidity values are given in brackets.

TABLE 16: FINAL MEAN CHEMSTOR ACIDITY VALUES DUE TO TEMPERATURE, CHEMSTOR APPLICATION RATES WITHIN MOISTURE AND MOISTURE CONTENT, PERCENT.

a) Temperature

Temperature conditions									
T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
0.41	0.72	0.40	0.76	0.40	0.42	0.73	0.43	0.66	0.42

b) Chemstor application rates within moisture.

Level of Chemstor applied	Initial Moisture content, percent		
	18.7	22.6	25.5
C_1	0.26 (0.28)	0.37 (0.39)	0.43 (0.52)
C_2	0.42 (0.36)	0.52 (0.51)	0.78 (0.60)
C_3	0.47 (0.49)	0.60 (0.68)	0.96 (0.82)

c) Moisture content

Initial Moisture content, percent		
18.7	22.6	25.5
0.38 (0.40)	0.49 (0.49)	0.72 (0.66)

5.3.1 Analysis of variance - final Chemstor acidity values.

The analysis of variance for the final Chemstor acidity values are given in table 17. The main effects due to temperature, moisture content and C/M were highly significant. The second order interaction temperature x moisture (T x M) also was highly significant.

TABLE 17: ANALYSIS OF VARIANCE - FINAL CHEMSTOR ACIDITY VALUES.

Source of Variation	Degrees of Freedom	Mean Square	F
R	1	0.0243	<1
M	2	1.7757	58.69**
C/M	6	0.6504	21.50**
Error (1)	8	0.0303	
T	9	0.4526	59.98**
TM	18	0.0315	4.17**
TC/M	54	0.0067	<1
Error (2)	81	0.0075	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

5.4 Analysis of variance - pH.

The analysis of variance for the final pH values (Appendix B) are given in table 18. The effects due to temperature and C/M were significant at the 0.01 probability level. The moisture content of the barley was significant at the 0.05 probability level.

TABLE 18: ANALYSIS OF VARIANCE - FINAL pH.

Source of Variation	Degrees of Freedom	Mean Square	F
R	1	0.0067	<1
M	2	0.3705	8.60*
C/M	6	0.7317	16.98**
Error (1)	8	0.0431	
T	9	0.7585	55.68**
TM	18	0.0104	<1
TC/M	54	0.0224	1.65
Error (2)	81	0.0136	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

5.5 Analysis of variance - moisture content.

The analysis of variance for the final moisture content is given in table 19. The main effect due to moisture was highly significant.

TABLE 19: ANALYSIS OF VARIANCE - FINAL MOISTURE CONTENT.

Source of Variation	Degrees of Freedom	Mean Square	F
R	1	15.78	4.20
M	2	630.47	167.90**
C/M	6	0.17	<1
Error (1)	8	3.76	
T	9	0.1177	1.07
TM	18	0.0717	<1
TC/M	54	0.1428	1.30
Error (2)	81	0.1097	

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

5.6 Analysis of variance - length of storage.

The mean length of storage due to C/M and temperature are given in table 20.

The analysis of variance for the length of storage is given in table 21. The main effects due to C/M and temperature were highly significant. The third order interaction temperature x C/M was also highly significant.

TABLE 20: MEAN LENGTH OF STORAGE DUE TO CHEMSTOR APPLICATION RATES WITHIN MOISTURE AND TEMPERATURE.

a) Chemstor application rates within moisture

Moisture Level	Chemstor application rate		
	C_1	C_2	C_3
M_1	95.5	150.0	150.0
M_2	113.0	150.0	141.0
M_3	104.0	141.0	150.0

b) Temperature

Temperature condition									
T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
107.8	145.0	110.0	150.0	126.0	145.0	150.0	145.0	138.9	108.0

TABLE 21: ANALYSIS OF VARIANCE -- LENGTH OF STORAGE.

Source of Variation	Degrees of Freedom	Mean Square	F
R	1	464.01	<1
M	2	195.95	<1
C/M	6	13016.9	12.79**
Error (1)	8	1017.80	
T	9	5722.90	15.40**
TM	18	619.41	1.67
TC/M	54	1492.00	4.01**
Error (2)	81	371.66	

* Significant at 0.05 probability level.
** Significant at 0.01 probability level.

5.7 Microbial examination.

The microflora were estimated per gram of grain rather than per gram of dry matter. The initial microbial load of the 10.0% moisture barley consisted of 800 moulds per gram and 1.18×10^6 bacteria per gram. The identification of the mould population was not carried out as it was assumed that these moulds would consist predominantly of field fungi and not storage fungi.

5.7.1 Mould content.

The *Aspergillus* species, consisting of three groups, namely, *A. ruber*, *A. flavus*, and *A. glaucus*, were the predominate mould isolated from the Chemstor treated high moisture barley. The relative abundance of each *Aspergillus* group was dependent on the rate of Chemstor application, moisture content and temperature. The *Penicillium*, *Mucor* and *Cladosporium* species were also isolated in trace quantities.

Appendix C gives the mould count data for all the units. Where applicable, the relative occurrence of each *Aspergillus* group is also given.

Figures 11, 12, 13 and 14 are plots of the relative occurrence of the three *Aspergillus* groups with respect to Chemstor application rates, moisture content and temperature. The points in each figure represent the averages of applicable units which are given in Appendix C.

5.7.1.1 Effect of Chemstor application rates.

At 12.5°C (figure 11), the *A. glaucus* group was the predominate mould at the lowest Chemstor application rate, C_1, within each moisture content. As the Chemstor application rates within each moisture content increased, the relative occurrence of *A. glaucus*

decreased. On the other hand, the A. *ruber* group increased in relative abundance with respect to increasing Chemstor application rate with each moisture content. Also, the relative abundance of the A. *flavus* group increased by 11% with increasing Chemstor application rates for the 18.9% and 22.5% moisture barley. However, at 25.5% moisture content, the relative abundance of the A. *flavus* group was 42% higher with respect to increased Chemstor application rates.

At 28.0°C (figure 12), the A. *glaucus* group decreased in relative abundance with increasing Chemstor application at 18.9% and 25.5%. There was a 6% increase in relative abundance of A. *glaucus* at the 22.5%. A 76% increase was found in the relative abundance of A. *ruber* with increasing Chemstor application rate at 18.9%. No trend could be obtained at the 22.5% moisture since only one point was present. At 25.5% moisture, the A. *glaucus* group increased very little - only 9% with respect to increasing Chemstor application rate. The A. *flavus* group indicated a drop in relative occurrence with increasing Chemstor application rate at the 18.9% and 22.5% moisture levels. However, at the 25.5% moisture level the relative occurrence of A. *flavus* increased with increasing Chemstor application.

5.7.1.2 Effect of moisture content.

At 12.5°C (figure 13), the optimum relative occurrence of A. *ruber* was at 22.5% moisture, and of A. *glaucus* and A. *flavus* at 25.5% moisture.

At 28.0°C (figure 14), the optimum relative occurrence of A. *ruber* was at 18.9% moisture, of A. *flavus* at 22.5% moisture, and of A. *glaucus* at 25.5% moisture.

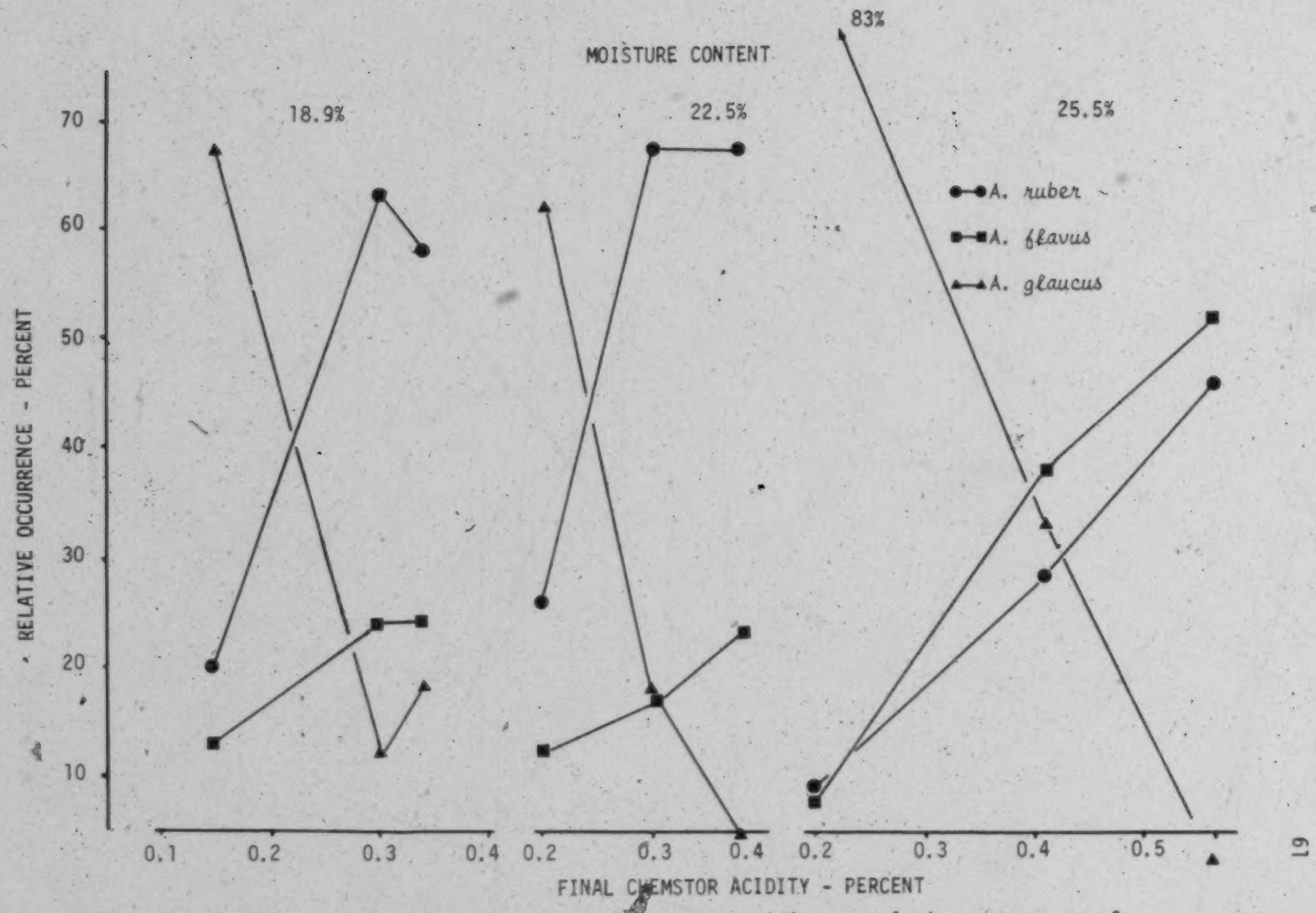

Figure 11: Graph illustrating the effect of Chemstor acidity on relative occurrence of the *Aspergillus* moulds at 12.5°C.

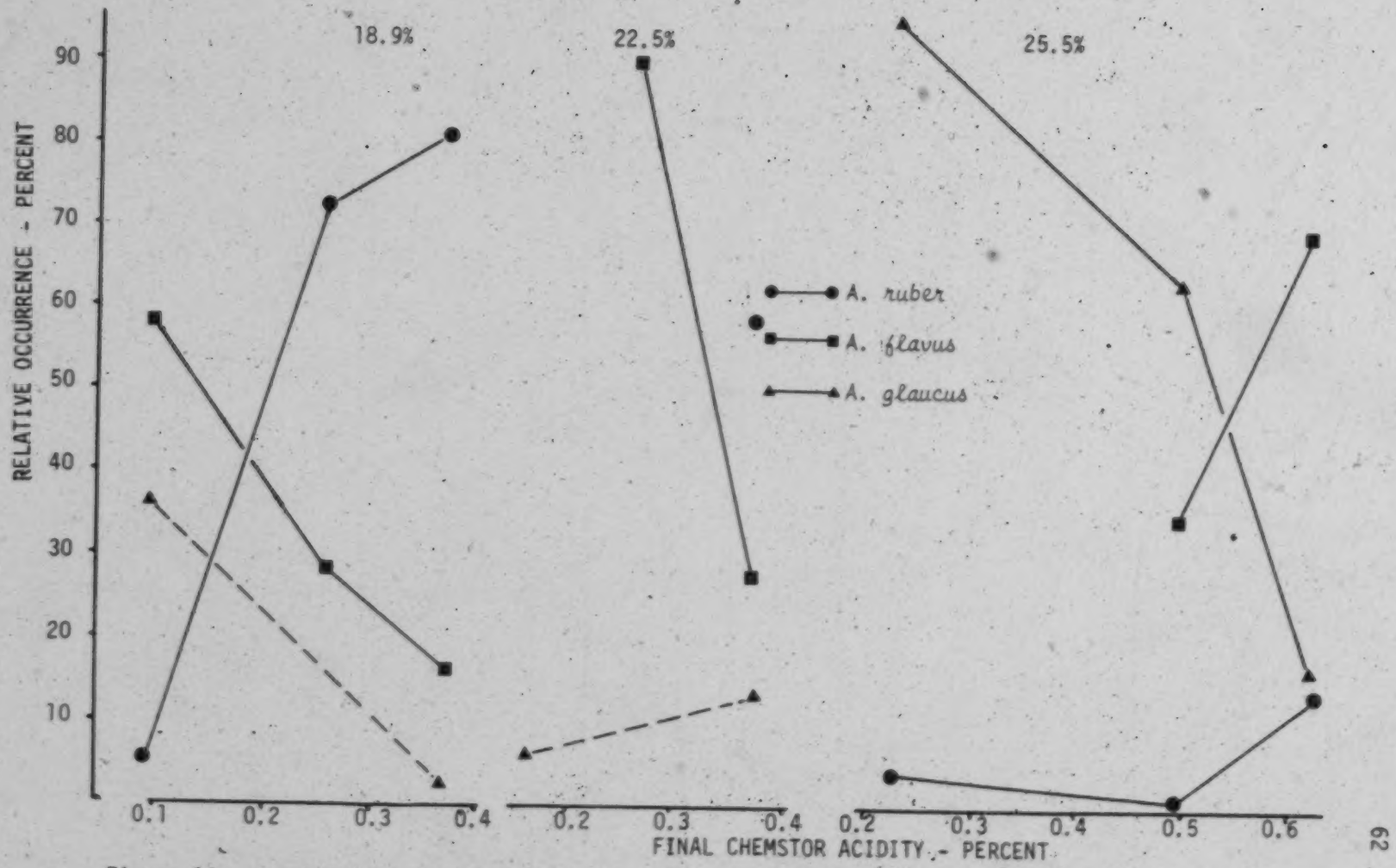

Figure 12: Graph illustrating the effect of Chemstor acidity on relative occurrence of the *Aspergillus* moulds at 28.0°C.

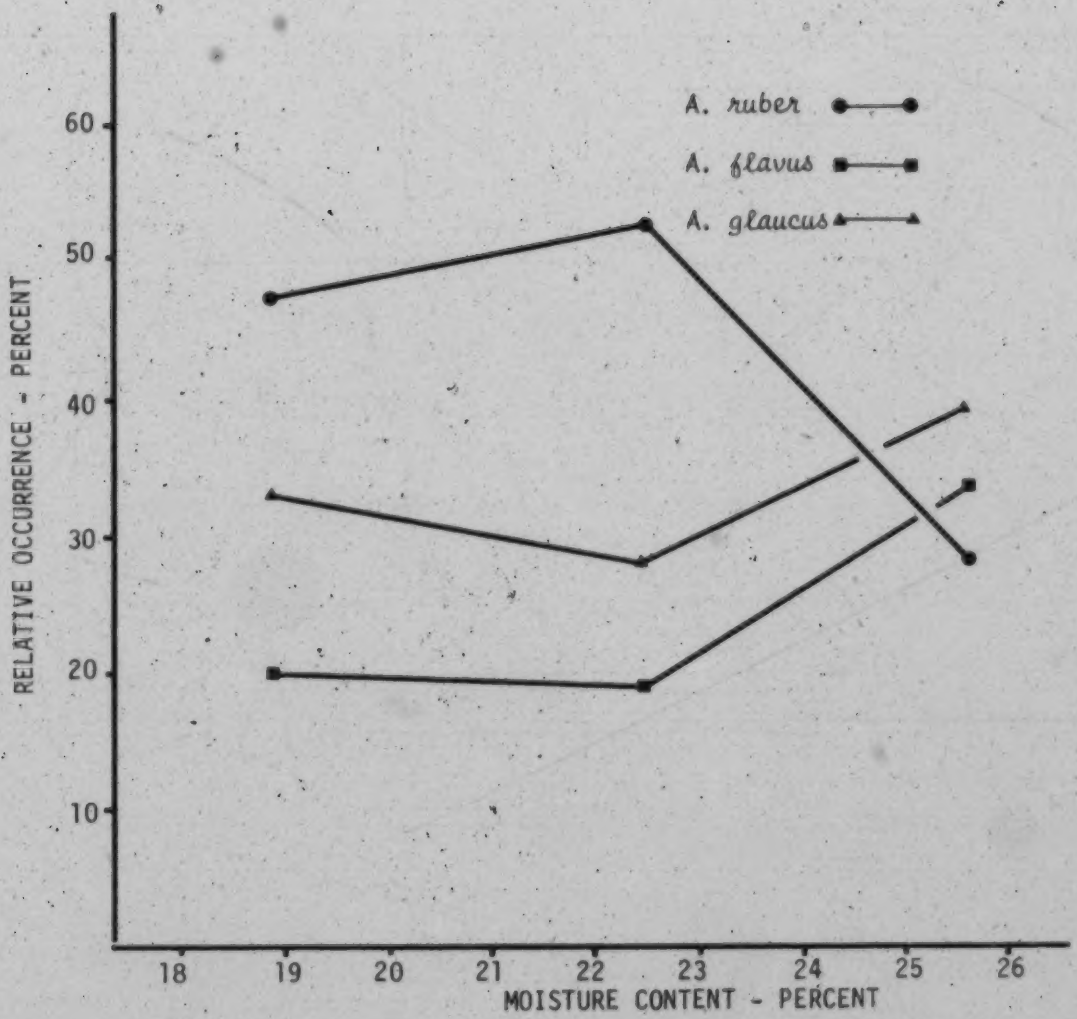

Figure 13: Graph illustrating the effect of moisture content on the relative occurrence of the *Aspergillus* moulds at 12.5 °C.

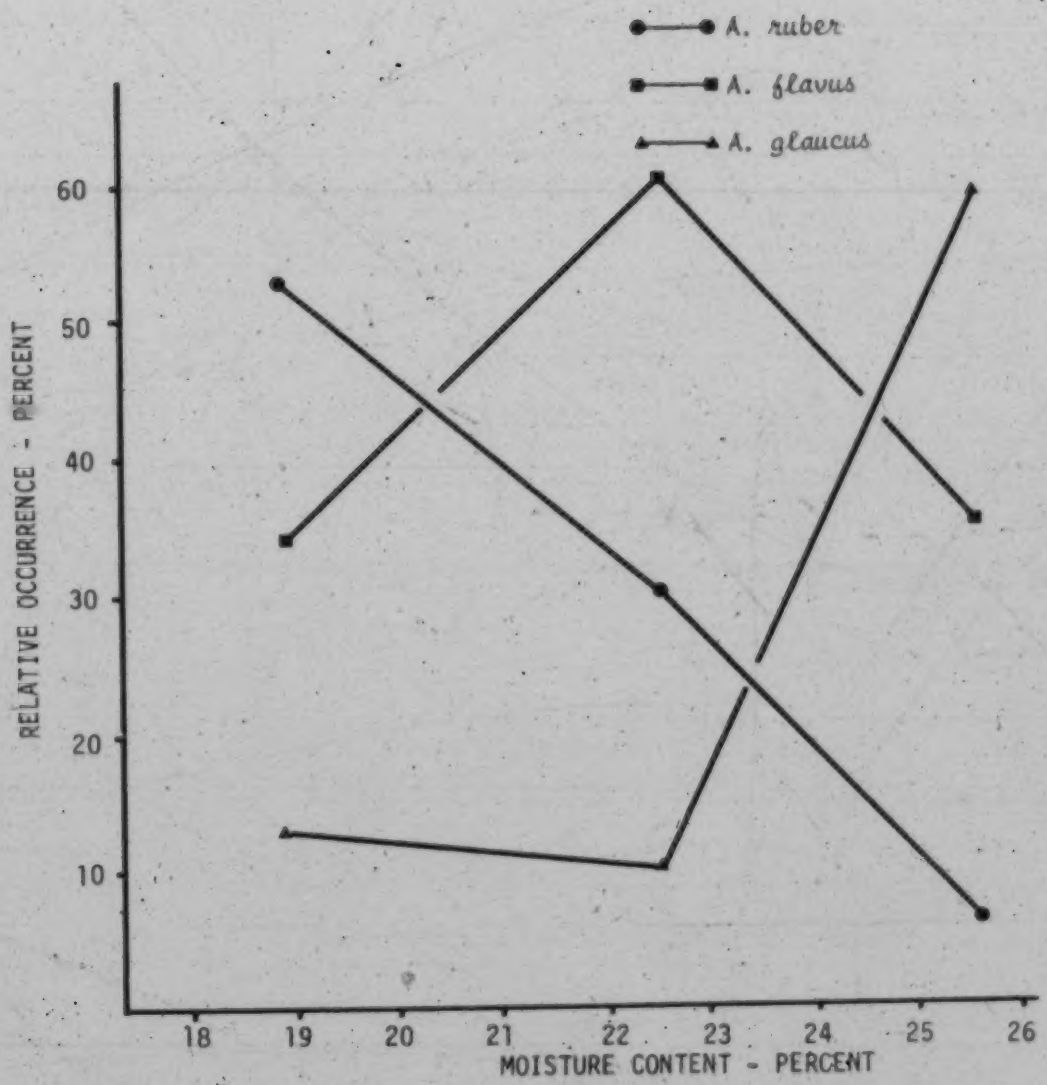

Figure 14: Graph illustrating the effect of moisture content on the relative occurrence of the *Aspergillus* moulds at 28.0°C

5.7.1.3 Effect of temperature.

Table 22 gives the relative occurrence of the *Aspergillus* groups due to temperature at various moistures.

TABLE 22: THE RELATIVE OCCURRENCE OF THE *Aspergillus* MOULDS DUE TO TEMPERATURE EFFECTS, PERCENT.

Final moisture content, percent	Mould group	Temperature, °C	
		12.5	28.0
18.9	*A. ruber*	47	53
	A. flavus	20	34
	A. glaucus	33	13
22.6	*A. ruber*	53	30
	A. flavus	19	60
	A. glaucus	28	10
26.5	*A. ruber*	28	6
	A. flavus	33	35
	A. glaucus	39	59

5.7.2 Bacterial count.

The bacterial population was greatly reduced due to the application of Chemstor to the high moisture barley. As a result, insufficient bacterial data (Appendix C) was obtained to indicate any trends. However, units $R_1M_1C_1T_5$ and $R_2M_1C_1T_5$ each yielded bacterial counts of 160,000 and 97,000, respectively at 50 days. Unit $R_1M_1C_1T_5$ was removed at 50 days due to mould proliferation while, unit $R_2M_1C_1T_5$ was maintained for the duration of the experiment.

6. DISCUSSION OF RESULTS

6.1 Carbon dioxide production.

Carbon dioxide production served as an indication of mould development for units treated at 41% and 51%, but not at 29%, of the recommended Chemstor application rate. The sequential decline of CO_2 production, at 41% and 51% treatment rates, to an average of 6 mg. CO_2 at 50 days (figure 9) suggests that the Chemstor application rates were sufficient to sterilize the grain kernels and prevent microflora from developing. However, in units where mould development occurred ($R_1M_1C_1T_9$, $R_1M_2C_3T_1$, $R_1M_2C_3T_{10}$, $R_2M_3C_1T_2$, $R_2M_3C_2T_3$ and $R_2M_3C_2T_{10}$), a sudden rise in CO_2 production was noted.

The high moisture barley treated at 29% of the recommended Chemstor application rate also resulted in decreased CO_2 production with time. However, at 50 days, no decrease in CO_2 production occurred. Visual inspection of units yielding high CO_2 production indicated no evidence of mould development.

Duncan's test on the mean CO_2 production rates for each temperature condition for all the CO_2 determination intervals are given in Appendix D. Of interest is the effect of the three storage temperatures, 12.5°C, 28.0°C and 37.0°C on the CO_2 production rate.

At 10 days, Duncan's Test indicated that no variable differences existed in the mean CO_2 production between units stored within either storage temperature (12.5°C and 28.0°C) but the mean CO_2 production from the units stored at 28.0°C differed significantly ($P < 0.05$) from that of units stored at 12.5°C. The units stored at 28.0°C produced more CO_2 than those stored at 12.5°C

At 20 days, the mean CO_2 production from the temperature

condition T_2, stored at 37.0°C, was significantly greater ($P < 0.05$) than the temperature condition T_1, stored at 28.0°C. The mean CO_2 concentration from the temperature condition T_6 decreased and was not significantly different ($P < 0.05$) from the mean CO_2 production from the temperature conditions T_3, T_4 and T_5. The mean CO_2 production from the temperature condition T_7, stored at 37.0°C, was not significantly different ($P < 0.05$) from the temperature conditions T_8, T_9 and T_{10}, stored at 28.0°C.

At 30 days, the mean CO_2 production from the temperature conditions T_1, T_3 and T_4 were significantly different ($P < 0.05$). Temperature conditions T_2, T_5, T_6, T_7 and T_8; and T_9 and T_{10} were not significantly different ($P < 0.05$).

As expected, Duncan's Test at 10, 20 and 30 days indicated that sudden changes in the storage temperature conditions increased or decreased the rate of CO_2 production. This was evident by the fact that the mean CO_2 production for each temperature condition was normally underscored once.

Subsequent comparison of the temperature conditions will be restricted to grouping the units as follow: T_5, T_6 and T_8 at 12.5°C; T_1, T_3 and T_{10} at 28.0°C; and T_2, T_4, T_7 and T_9 at 37.0°C.

At 40 days, no differences in the mean CO_2 production existed between the units stored at 12.5°C and 37.0°C. The mean CO_2 production from the units T_1, T_3 and T_{10}, stored at 28.0°C, were all significantly different $P < 0.05$) from each other.

For the CO_2 determination intervals 50 to 150 days, generally, the mean CO_2 production between units within each storage temperature was not different. Deviations from the general statement

occurred at the 60, 70 and 80 day CO_2 determination interval for unit T_5 stored at 12.5°C and unit T_{10} stored at 28.0°C for 125 days.

The examination of the CO_2 production data did not indicate why the mean CO_2 production from temperature condition T_5 was different from T_6 and T_8. The production of 200 mg.CO_2/100 grams dry matter/ 25 days in unit $R_1M_2C_3T_{10}$ resulted in temperature condition T_{10} being significatnly different ($P < 0.05$) from temperature conditions T_1 and T_3 at the 125-day CO_2 determination interval. Mould proliferation also was evident in unit $R_1M_2C_3T_{10}$. Unit $R_1M_2C_3T_1$ also produced moulds, paralleled with 164 mg. CO_2/100 grams dry weight/ 25 days at the 125-day CO_2 determination interval.

6.2 Final Chemstor acidity.

Duncan's Test on the final mean Chemstor acidity due to temperature (Appendix D) indicated that the units stored at 37.0°C yielded a higher final mean Chemstor acidity than those stored at 12.5°C and 28.0°C. There were no differences between the final mean Chemstor acidities at 12.5°C and 28.0°C.

Table 23 gives the mean difference between the final and initial Chemstor acidity for each temperature condition. There was a decrease in acidity in the units stored at 12.5°C and 28.0°C. The decrease was probably due to the vaporization of the Chemstor during aeration. At 37.0°C all units exhibited an increase in Chemstor acidity. The actual acidity increase at 37.0°C was probably higher since the amount of Chemstor lost due to vaporization was not measured.

TABLE 23: COMPARISON OF THE OVERALL INITIAL AND FINAL CHEMSTOR ACIDITY FOR EACH TEMPERATURE CONDITION.

	Storage temperature, °C									
	12.5°C			28.0°C			37.0°C			
	T_5	T_6	T_8	T_1	T_3	T_{10}	T_2	T_4	T_7	T_{10}
Final mean Chemstor acidity	0.40	0.42	0.43	0.41	0.40	0.42	0.72	0.76	0.73	0.66
Overall Initial Chemstor acidity	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
Difference	-0.12	-0.10	-0.09	-0.11	-0.12	-0.10	+0.20	+0.24	+0.21	+0.14

Duncan's Test on the final mean Chemstor acidity due to Chemstor application rate within moisture (Appendix D) showed that the Chemstor levels C_2 and C_3 at 18.7% and 22.6% moisture were not significantly different ($P < 0.05$). The Chemstor levels C_1, C_2 and C_3 at 25.5% moisture were all significantly different ($P < 0.05$).

6.3 Length of storage.

Duncan's Test (Appendix D) showed that on the mean length of storage due to C/M no differences existed ($P < 0.05$) between Chemstor levels C_2 and C_3 within each moisture. Chemstor level C_1 was less effective and different from C_2 and C_3.

Duncan's Test on the mean length of storage due to temperature indicated that no difference existed ($P < 0.05$) between units stored at 28.0°C. No differences existed between the units stored at 12.5°C and 37.0°C.

6.4 Microflora.

The Chemstor application rates combined with the fixed temperature conditions limited the development of moulds within the high moisture barley. For example, 74% of the units yielded less than

300 moulds per gram during all mould determination intervals, while 26% of the units yielded mould counts greater than 300 in at least one or more mould determinations. Furthermore, in the units yielding a mould count greater than 300, 18% were treated with Chemstor level C_1, and 8% were treated with Chemstor level C_2 and C_3; all units were stored at either 12.5°C or 28.0°C. As a result, statistical analysis of the mould data was not carried out since visual inspection yielded the desired information.

6.4.1 Effect of temperature.

The *A. glaucus*, *A. ruber* and *A. flavus* mould continued to persist at 12.5°C and 28°C (figures 11 and 12). Although their numbers were reduced, the presence of moulds indicates that spoilage may be initiated some time in the future. Units stored at 37.0°C exhibited virtually no moulds during the test periods. The exceptions being that units $R_1M_1C_1T_9$, $R_2M_1C_1T_9$ and $R_2M_3C_1T_2$ had some mould development.

6.5 Summary of results.

In this investigation an attempt was made to study the biochemical and storage characteristics of high moisture barley treated with marginal levels of Chemstor. The results indicated that biochemical changes and duration of storage were influenced by the experimental conditions. However, several queries may arise from this investigation.

Firstly, no consideration was given to the degree of the initial microbial infection. The barley used in this investigation initially had a viable count of 1,180,000 bacteria and 800 moulds per gram based on 10.0% moisture, which may be relatively low. In this

investigation, the bacterial population was nearly eliminated, however, the storage moulds continued to persist especially at the 12.5°C storage temperature. If the initial mould contamination of the barley had been higher, then the high moisture barley may have spoiled at a faster rate and the levels of Chemstor applied may have been insufficient to hinder such spoilage.

Secondly, counts of bacteria and moulds, especially in units $R_1M_2C_3T_1$, $R_1M_2C_3T_{10}$, $R_2M_3C_2T_{10}$, $R_2M_3C_2T_3$, $R_2M_3C_1T_2$ and $R_2M_1C_1T_9$, were difficult to obtain. Localized moulding occurred in these units and a representative sample could not be obtained. Examination of the storage data revealed that moulding occurred shortly after the 50-day or 100-day microbial analysis. Therefore, the possibility of external contamination of these units existed. Furthermore, it was also probable that the units may have become entirely moulded if left in storage for sufficient time.

Thirdly, the techniques used for measuring Chemstor acidity were adequate for the initial acidity, but were considered inadequate for the final acidity. Final acidities were recorded as Chemstor acidity with no knowledge of the absolute amount of Chemstor remaining on the kernels, nor the amount of acidity attributable to biochemical changes. Although there was an overall decrease in the final Chemstor acidity at 12.5°C and 28.0°C, an increase in the final Chemstor acidity was noted at 37.0°C (table 23). From the data, it may be assumed that an increase in acidity occurs at 37.0°C, but the same assumption cannot be made for the 12.5°C and 28.0°C storage temperatures without the determination of the absolute amount of Chemstor remaining on the kernels.

Fourthly, although orthogonality was maintained in the

analysis of variance by the substitution of data and the elimination of an entire Chemstor application level, the use of a common storage period for the analysis of final pH, Chemstor acidity and moisture content was not considered. Generally, the elimination of a mouldy unit resulted in final pH and Chemstor acidity values being higher than from those units stored in similar experimental conditions for the duration of the experiment. The extrapolation of data, for the units removed, to a common basis of 150 days was not considered practical since the final pH and final Chemstor acidity could not be predetermined.

Fifthly, a thorough investigation of the physical characteristics such as odor and kernel color were not considered. However, a few points are noteworthy. The grain kernels, from the units stored at 37.0°C, turned notably darker. No color differences were noted between the units stored at 12.5°C and 28.0°C. Units stored at 12.5°C and 28.0°C emitted a Chemstor odor, whereas, no odor was emitted from the units stored at 37.0°C.

7. CONCLUSIONS

Under the conditions of the experiment described, the following conclusions are made:

1. Carbon dioxide production from chemically treated high moisture barley can be used as an indicator of microbial development under laboratory conditions. However, this method should be restricted to high moisture grain initially treated with sufficient chemical preservative to sterilize the grain kernels. Subsequent sudden rises in carbon dioxide production from the treated high moisture barley may be attributed to microbial development.

2. The *Aspergillus ruber*, *A. Flavus* and *A. glaucus* moulds can persist in high moisture barley treated with reduced levels of Chemstor. The relative abundance of each mould was influenced by the Chemstor application rate, moisture content and storage temperature.

3. Virtually no moulds and bacteria were found at a storage temperature of 37.0°C regardless of the Chemstor application rates. Microfloral counts were obtained at 12.5°C and 28.0°C storage temperatures with greater numbers of microflora occurring at the lower temperature. Increasing microfloral counts were obtained from barley treated with decreasing Chemstor application rates. The data suggest that Chemstor may be used more effectively at higher temperatures, provided that the chemically treated high moisture grain is initially stored at a high temperature.

4. The application of Chemstor at 41% and 51% of the commercially recommended rate to 18 and 25% moisture barley reduced the numbers of bacteria and moulds and maintained the keeping quality of the grain effectively for up to the experimental period of 150 days.

5. Barley stored at 37.0°C exhibited a higher total acidity content than that stored at 12.5°C or 28.0°C. The increase in acidity probably could be attributed to the formation of lactic acid.

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9. APPENDICES

APPENDIX A: CARBON DIOXIDE PRODUCTION.

Carbon dioxide production data collected for all the units are presented in the following pages.

In the heading to each section the Moisture level, Chemstor application level and Replicate number are given. The Moisture and Chemstor levels correspond to the obtained initial experimental levels, which are given in table 11.

The columns in each section correspond to the carbon dioxide production determination interval, which are appropriately identified (days). The rows in each section (numbered one to ten) correspond to the imposed experimental conditions. Reference may be made to table 8 for the row identification.

Asterisks identify the time of removal of a unit from the experimental condition. Where applicable, the carbon dioxide production data proceeding an asterisk is comprised of the average from units in the same experimental condition. The carbon dioxide production data is given as mg. CO_2/100 grams dry matter/unit time.

	10	20	30	40	50	60	70	80	90	100	125	150
	Moisture 1			Chemstor level 1			Replicate 1					
1	12.7	154.5	187.5	133.4	112.4	92.2	90.0*					
2	46.3	312.3	24.2	6.0	4.3	12.3	9.2	6.6	6.0	6.2	17.0	12.2
3	49.0	31.9	160.6	160.8	135.3	106.0*						
4	46.3	25.8	273.4	6.0	5.2	9.2	9.2	6.6	7.3	7.4	15.2	12.3
5	41.3	29.6	27.5	22.0	23.0	70.0*						
6	173.7	20.0	23.1	23.0	24.7	24.0	88.3*					
7	175.5	194.5	17.0	10.0	14.8	9.2	2.0	3.6	7.1	7.1	15.8	10.3
8	159.6	154.5	24.2	30.0	39.7	50.0	70.0*					
9	161.8	170.0	177.9	20.0	64.0*							
10	175.1	172.2	137.5	102.4	45.0	106.0*						
	Moisture 1			Chemstor level 2			Replicate 1					
1	56.4	177.3	107.0	35.0	10.3	3.1	4.5	2.4	2.3	2.3	7.3	6.7
2	60.1	182.9	13.1	7.6	7.6	6.7	7.0	7.7	1.7	1.0	9.7	11.0
3	54.2	33.7	148.4	78.4	24.3	8.2	5.5	2.0	2.5	3.6	26.1	88.2
4	54.2	33.7	165.0	10.0	9.0	6.7	9.4	9.4	4.3	4.9	18.8	15.8
5	56.4	38.1	27.6	3.8	1.8	17.6	17.6	17.6	14.0	14.0	21.3	17.0
6	189.2	28.2	19.3	14.0	14.0	9.1	9.1	9.1	6.0	5.2	8.5	4.9
7	111.3	108.2	12.7	7.4	7.6	5.6	11.6	10.2	3.3	3.3	17.0	11.6
8	191.4	113.1	6.6	6.8	6.8	2.0	2.0	2.0	3.0	3.1	11.6	1.2
9	198.5	113.2	51.9	10.0	9.0	5.6	6.8	6.9	7.1	7.1	17.0	12.2
10	205.8	133.0	58.5	13.1	7.2	2.9	6.6	8.1	3.3	3.3	3.0	6.7
	Moisture 1			Chemstor level 3			Replicate 1					
1	33.9	121.0	65.7	25.9	9.0	3.1	2.8	2.4	2.6	2.5	6.7	6.1
2	6.8	108.6	10.4	10.0	6.7	5.6	8.0	8.3	6.1	6.1	17.6	12.2
3	28.5	25.8	123.7	53.7	6.7	8.2	2.8	2.8	3.3	3.3	6.7	6.1
4	14.9	31.8	149.1	15.0	9.0	6.7	6.0	6.7	7.1	7.1	15.8	13.4
5	39.3	25.8	21.9	22.0	22.1	18.0	10.0	9.6	10.0	11.8	19.5	11.6
6	152.3	20.3	12.0	8.0	7.7	7.1	7.1	7.1	10.0	12.2	12.8	5.5
7	160.8	70.5	12.0	10.0	7.6	6.7	7.0	8.2	4.8	4.9	17.0	13.4
8	128.1	77.0	9.3	3.1	3.8	1.8	1.8	2.0	2.8	2.8	3.0	4.3
9	150.2	85.8	51.9	10.0	1.0	7.7	2.8	2.8	6.1	6.1	16.4	11.0
10	145.5	70.4	37.2	10.7	32.8	3.1	3.0	2.6	0.5	0.9	6.1	6.7

	10	20	30	40	50	60	70	80	90	100	125	150
	Moisture 1		Chemstor level 1			Replicate 2						
1	13.5	93.0	78.6	38.9	37.4	110.4*						
2	18.4	214.6	20.8	4.0	5.8	5.0	6.1	6.1	6.1	6.1	14.5	10.2
3	18.9	14.6	91.4	75.1	59.4	115.4*						
4	16.8	13.8	215.9	16.3	5.8	10.0	5.5	5.4	5.4	5.4	13.3	11.9
5	15.1	10.8	9.5	9.0	8.9	12.5	7.3	7.3	7.3	7.3	24.2	4.5
6	93.5	14.6	11.2	11.0	10.3	11.5	8.6	8.6	8.6	8.6	23.0	30.1
7	98.9	154.8	15.5	11.5	8.9	5.0	4.4	4.4	4.4	4.4	13.9	10.2
8	89.0	75.7	16.9	10.0	4.9	10.0	7.0	7.0	7.0	7.0	19.3	17.0
9	96.8	81.1	122.8	9.3	15.6	91. *						
10	95.1	82.2	60.9	44.6	87.4*							
	Moisture 1		Chemstor level 2			Replicate 2						
1	11.9	93.5	59.2	21.2	11.1	7.0	3.8	3.8	3.8	3.8	5.4	3.7
2	15.1	146.5	10.6	4.0	5.8	4.6	6.8	8.3	8.3	10.0	13.9	8.5
3	16.2	13.0	84.3	41.5	21.8	10.0	1.9	1.7	1.7	1.7	5.4	2.8
4	17.3	9.7	117.9	7.5	4.0	3.5	4.0	6.5	6.6	8.0	8.5	6.2
5	17.3	12.4	13.0	10.0	4.0	2.8	6.0	7.0	7.0	7.2	15.7	7.4
6	100.0	13.0	10.0	7.7	4.9	3.1	4.0	6.5	6.6	8.0	9.7	4.0
7	100.6	90.8	4.9	0	2.7	3.0	7.0	7.1	7.1	7.1	12.1	11.9
8	101.6	62.7	8.0	6.1	4.9	6.0	7.0	6.5	6.5	6.8	7.3	2.3
9	103.8	67.0	57.0	9.3	7.1	6.9	6.9	6.9	7.0	7.0	10.9	8.5
10	91.4	56.2	35.8	20.3	5.8	7.0	0	0	0	0.5	5.4	4.5
	Moisture 1		Chemstor level 3			Replicate 2						
1	12.9	73.0	40.2	15.9	6.2	3.2	3.0	3.1	3.1	3.1	5.4	6.8
2	10.3	82.5	7.5	7.1	4.0	2.7	2.6	2.6	2.6	2.6	9.1	5.7
3	14.0	10.8	69.8	23.0	12.0	5.3	4.4	4.9	4.9	4.9	5.4	6.8
4	11.3	9.2	88.3	6.6	8.0	5.5	5.3	5.3	5.3	5.3	12.1	2.8
5	12.9	9.7	0	0	4.0	4.5	4.7	4.6	4.6	4.7	9.1	32.4
6	79.9	10.8	6.0	5.5	4.0	3.2	3.0	3.0	3.0	3.0	5.4	1.7
7	87.9	68.1	7.9	5.3	8.9	3.7	3.0	3.0	3.0	3.0	10.3	17.6
8	80.9	36.2	6.3	3.0	0	1.0	1.3	1.3	1.3	1.6	5.4	4.5
9	80.4	46.2	37.1	7.5	4.0	3.3	3.3	3.3	3.3	3.6	8.5	5.7
10	82.0	45.4	22.1	11.9	7.1	1.9	1.8	1.8	1.8	1.8	7.9	8.5

	10	20	30	40	50	60	70	80	90	100	125	150
	Moisture 2		Chemstor level 1				Replicate 1					
1	131.9	270.7	115.0	51.0	56.6	121.5*						
2	137.1	350.2	13.1	11.7	12.7	12.3	8.0	8.0	8.0	8.5	22.2	8.8
3	137.6	73.4	214.6	119.2	122.1*							
4	132.9	79.1	248.5	14.0	17.4	14.0	8.0	6.3	3.0	2.3	24.5	15.3
5	129.1	80.3	57.0	30.0	23.7	64.9	47.1*					
6	286.6	39.6	25.2	15.2	15.0	9.7	9.0	6.9	8.1	9.0	11.7	5.3
7	334.3	175.1	11.2	8.4	10.4	14.9	2.9	1.6	1.6	1.6	7.0	5.7
8	349.0	150.8	22.9	10.0	7.9	7.0	6.6	5.9	6.5	6.8	8.1	5.1
9	314.4	129.2	63.1	10.8	12.7	10.1	10.7	9.6	10.0	10.3	21.7	17.5
10	298.2	144.5	65.5	25.2	80.6	112.0*						
	Moisture 2		Chemstor level 2				Replicate 1					
1	81.1	193.4	62.2	15.4	5.2	5.3	3.1	3.0	3.2	3.2	10.6	5.9
2	98.6	166.0	11.7	10.8	10.4	11.0	11.5	11.6	11.5	11.4	22.9	18.9
3	74.1	37.2	132.3	42.5	15.1	10.3	5.0	5.5	5.0	4.6	12.9	7.3
4	94.8	45.2	22.6	10.3	12.7	12.7	12.6	12.0	10.0	8.7	23.4	18.3
5	90.1	41.2	30.9	11.2	10.0	8.3	8.3	8.3	8.3	8.3	15.2	6.3
6	233.0	25.2	12.2	6.3	5.0	5.0	5.0	4.0	5.0	5.0	4.7	3.6
7	223.8	78.6	14.0	10.3	10.4	13.1	13.1	13.1	10.0	9.7	21.7	16.9
8	233.0	72.1	20.1	2.5	2.2	2.5	2.5	2.5	2.5	2.5	4.7	3.6
9	221.5	67.7	33.2	9.8	12.7	11.0	11.1	12.0	10.0	9.7	22.2	17.0
10	221.5	84.1	31.8	3.7	7.5	5.3	3.7	3.0	3.0	4.5	12.3	6.1
	Moisture 2		Chemstor level 3				Replicate 1					
1	56.5	101.7	43.5	15.0	7.1	8.0	8.0	11.7	28.5	26.9	164.3*	8.0
2	57.0	104.6	11.7	1.0	9.4	11.7	11.0	12.0	11.9	10.0	24.5	15.3
3	56.0	27.4	63.2	35.2	15.1	5.3	2.1	2.1	2.1	2.1	10.6	14.1
4	56.0	25.1	81.3	8.5	9.8	12.8	13.9	12.0	10.0	11.9	20.5	14.3
5	55.1	16.0	20.1	9.0	6.6	5.4	5.4	5.4	5.4	5.4	6.5	3.0
6	142.7	13.7	8.9	4.0	3.5	3.3	3.3	3.3	3.3	3.3	3.8	3.8
7	117.4	58.9	9.4	10.3	12.7	11.0	10.5	10.0	8.6	9.0	21.7	15.6
8	140.4	41.3	17.8	8.6	7.0	3.0	3.0	2.3	2.0	2.0	8.1	4.5
9	142.7	45.1	29.4	11.2	12.7	13.2	7.1	13.1	9.0	8.1	27.5	16.7
10	133.5	48.0	27.6	2.8	8.5	12.3	12.3	12.2	20.0	55.2	91.7*	8.0

	10	20	30	40	50	60	70	80	90	100	125	150
	Moisture level 2			Chemstor level 1			Replicate 2					
1	86.7	223.6	91.7	20.0	23.5*							
2	86.7	290.1	15.7	11.1	7.0	10.4	7.0	6.4	6.3	6.3	16.6	15.6
3	24.4	19.9	158.1	49.8	92.8*							
4	91.2	52.7	207.9	12.4	8.4	10.4	9.0	9.3	9.0	9.4	17.2	12.6
5	90.7	58.3	12.0	10.0	8.2	17.2	11.0	11.6	11.6	12.2	53.0*	
6	268.5	34.2	18.2	7.0	5.0	5.2	3.0	0.5	1.5	1.3	5.7	3.0
7	279.8	124.7	15.2	12.0	11.2	12.0	9.0	8.0	8.0	9.0	14.0	13.2
8	282.1	150.7	16.0	9.0	4.7	3.4	3.1	3.1	3.1	3.1	10.2	7.8
9	279.8	135.4	36.4	12.4	8.4	10.4	8.2	8.2	8.2	8.2	16.0	12.0
10	279.8	136.0	29.5	12.4	7.4	13.0*						
	Moisture 2			Chemstor level 2			Replicate 2					
1	66.4	157.6	60.0	13.8	4.7	7.8	3.2	3.0	2.8	3.4	7.0	6.6
2	65.8	185.6	12.0	8.3	11.2	10.0	9.0	6.1	9.0	10.0	16.0	22.8
3	67.5	33.5	138.7	46.6	18.6	5.2	3.2	3.0	3.2	3.1	8.3	4.8
4	67.5	39.1	134.1	9.7	6.5	8.7	8.7	8.7	8.7	8.7	12.8	13.8
5	56.3	36.8	25.7	19.0	13.0	9.5	9.5	9.5	9.5	9.5	14.0	25.8
6	223.6	25.3	15.0	5.0	3.3	3.5	3.5	3.5	3.5	3.5	5.7	3.0
7	219.1	87.8	10.6	8.3	9.8	8.1	8.0	8.0	8.0	8.0	14.0	15.0
8	228.1	80.4	6.0	3.0	2.2	5.2	2.0	2.0	2.0	2.0	2.6	3.0
9	225.9	83.3	27.2	8.3	4.7	8.0	8.0	7.8	7.8	7.8	17.2	10.2
10	228.1	86.1	16.6	5.1	4.7	5.2	2.2	2.2	2.2	2.2	17.9	4.8
	Moisture level 2			Chemstor level 3			Replicate 2					
1	45.7	110.6	39.2	8.8	0.0	3.1	3.1	3.2	3.2	3.2	7.0	7.8
2	48.0	103.8	11.1	8.3	4.7	8.8	8.8	8.8	8.9	8.8	21.7	15.6
3	47.7	23.0	71.0	33.2	9.8	3.9	3.5	3.5	3.5	3.5	8.3	6.6
4	45.7	28.1	73.3	12.4	7.0	10.0	9.0	6.1	9.0	10.0	21.1	6.0
5	42.9	31.8	2.0	1.7	0.7	4.9	5.0	5.0	5.0	5.0	8.9	1.2
6	156.3	16.6	9.9	3.0	2.0	1.7	1.7	1.7	1.7	1.7	4.5	4.5
7	140.5	54.2	9.2	8.3	12.6	8.9	8.8	8.8	8.8	8.8	16.6	15.0
8	140.5	49.1	4.0	2.0	1.4	1.7	1.7	1.7	1.7	1.7	3.4	3.0
9	131.4	41.2	24.4	12.0	11.2	8.6	8.3	8.3	8.3	8.3	17.9	10.2
10	151.8	46.8	16.6	3.7	29.3	3.1	3.1	3.1	3.0	3.0	5.7	3.0

	10	20	30	40	50	60	70	80	90	100	125	150
	Moisture 3		Chemstor level 1				Replicate 1					
1	193.5	165.4	91.3	93.2	105.7	64.2*						
2	480.9	246.0	13.0	12.5	12.7	15.9	15.4	5.1	15.0	11.0	31.4	22.2
3	168.8	76.5	125.2	97.1	112.5	111.3*						
4	148.4	72.4	104.3	13.9	14.6	11.5	15.4	13.5	13.0	13.0	30.2	23.4
5	190.2	77.1	32.0	30.0	29.7	148.0*						
6	383.2	47.1	30.0	14.5	14.0	14.8	5.5	2.2	1.1	1.1	6.3	4.1
7	360.5	79.4	17.3	12.5	12.7	7.7	6.0	10.0	12.0	12.5	23.4	22.3
8	379.5	112.3	10.0	5.4	4.9	1.5	1.2	1.5	1.5	1.5	2.5	3.4
9	379.5	114.2	31.2	13.9	14.6	12.6	13.7	17.6	17.0	27.4	26.3	21.3
10	384.2	117.7	30.8	77.4	62.3	86.6*						
	Moisture 3		Chemstor level 2				Replicate 1					
1	57.0	53.6	12.0	9.6	5.4	2.7	2.7	3.4	6.0	6.0	13.2	5.7
2	57.7	112.3	11.1	13.0	11.2	15.2	15.2	15.2	15.2	15.2	32.0	20.2
3	54.6	18.8	30.1	9.6	5.4	5.5	2.7	2.3	5.0	6.0	14.4	9.1
4	52.2	19.4	40.4	11.5	10.7	14.4	14.4	14.4	14.4	14.4	32.0	24.8
5	65.5	20.0	13.0	5.3	4.9	2.5	2.4	2.4	2.4	2.4	4.4	3.5
6	99.6	8.8	3.3	2.0	4.9	2.3	2.2	2.3	2.3	2.3	8.2	3.6
7	102.0	36.5	12.5	12.5	8.8	5.5	15.5	15.5	15.5	15.5	28.8	22.1
8	106.7	25.9	2.8	2.0	4.9	1.8	1.6	1.6	1.6	1.6	3.8	3.9
9	73.5	27.7	15.4	16.8	8.8	1.7	1.0	19.0	30.1	35.3	26.3	24.0
10	109.1	32.4	11.1	4.3	5.4	2.7	3.4	4.0	4.0	4.0	12.6	13.1
	Moisture 3		Chemstor level 3				Replicate 1					
1	50.9	37.7	8.2	4.3	5.4	2.7	5.5	6.3	5.3	5.3	12.6	8.4
2	38.3	49.9	10.1	9.1	10.7	12.5	12.5	12.5	12.5	12.5	24.5	19.9
3	32.5	14.1	18.3	5.3	5.4	2.7	2.7	3.4	6.0	6.0	12.6	9.0
4	49.5	10.0	32.7	13.9	10.7	12.0	13.2	17.0	17.0	17.0	30.8	22.8
5	48.0	8.8	4.2	3.0	4.9	1.9	1.8	1.6	1.7	1.7	10.1	4.5
6	87.8	8.8	3.2	3.0	4.9	1.2	1.2	1.2	1.2	1.2	4.4	8.1
7	80.6	21.8	5.8	12.0	14.1	14.1	14.1	14.1	14.1	14.1	28.8	23.4
8	75.9	20.0	5.0	0.0	4.9	2.0	2.0	2.0	2.0	2.0	5.0	7.4
9	85.4	21.8	11.5	9.1	10.7	15.0	16.5	16.0	16.0	16.0	30.2	25.4
10	90.1	25.3	4.3	4.3	5.4	2.7	4.0	3.9	3.9	3.9	10.1	10.2

	10	20	30	40	50	60	70	80	90	100	125	150
	Moisture 3		Chemstor level 1			Replicate 2						
1	117.9	240.1	53.7	85.7*	120.0							
2	174.2	170.8	55.2	60.4	38.6	57.2*						
3	174.2	77.4	166.0	121.3	160.0							
4	155.8	72.1	103.6	13.6	11.7	12.7	8.2	8.2	8.2	8.2	19.1	15.6
5	159.4	90.5	50.0	40.0	36.5	76.2*						
6	271.0	89.1	30.0	17.0	7.2	3.9	2.4	2.2	2.2	2.2	1.5	0.0
7	96.0	83.2	17.4	19.0	20.5	10.3	9.0	9.0	9.0	9.0	22.5	22.8
8	350.5	140.8	15.0	4.0	2.5	2.4	1.2	1.0	1.0	1.0	0.0	3.0
9	282.7	136.0	26.1	7.8	13.2	10.3	8.1	8.1	8.0	8.1	23.9	21.0
10	356.7	148.6	22.7	48.2	76.7*							
	Moisture 3		Chemstor level 2			Replicate 2						
1	60.4	20.3	8.7	5.4	4.9	0.0	1.7	4.7	4.7	3.7	9.3	9.6
2	64.6	55.2	11.6	12.2	10.7	6.8	6.3	6.3	6.3	6.3	25.9	12.6
3	55.7	20.3	25.2	3.9	1.0	0.0*	2.6	3.6	4.9	4.9	12.2	9.4
4	58.7	22.3	37.7	15.6	13.7	10.4	10.3	10.3	10.3	10.3	27.9	25.8
5	52.7	24.2	8.0	4.0	2.2	1.5	1.5	1.5	1.4	1.5	0.0	9.0
6	128.9	50.3	7.0	4.0	2.2	3.0	3.5	3.6	3.6	3.8	13.0	25.8
7	115.3	37.3	13.1	12.7	14.7	11.8	11.6	11.6	11.6	11.6	30.0	19.8
8	122.1	36.3	17.9	5.4	7.8	1.5	1.5	1.5	1.4	1.5	3.8	0.0
9	115.3	38.7	11.6	12.7	10.7	13.4	13.4	13.4	13.4	13.4	23.9	19.8
10	106.3	37.3	0.0	5.4	8.3	91.3*	2.6	3.6	4.9	4.9	12.2	9.4
	Moisture 3		Chemstor level 3			Replicate 2						
1	38.1	7.3	6.6	5.4	0.0	4.0	5.2	4.6	4.6	4.9	13.6	10.8
2	30.9	33.1	14.0	14.6	15.6	12.6	12.2	12.2	12.2	12.2	32.0	28.0
3	39.3	8.8	10.2	4.4	0.0	4.0	5.4	5.0	4.7	4.7	13.6	10.8
4	37.5	8.3	21.3	14.6	14.7	12.6	12.2	12.2	12.2	12.2	29.3	25.8
5	35.7	9.2	3.5	2.5	1.8	1.2	1.2	1.2	1.1	1.1	0.0	3.0
6	57.1	5.3	3.5	2.5	1.8	1.2	1.2	1.2	1.1	1.1	0.0	0.0
7	60.7	26.7	16.0	12.2	17.6	12.5	12.5	12.5	12.5	12.5	31.4	24.0
8	50.0	10.7	3.5	2.5	1.8	1.3	1.2	1.3	1.3	1.3	0.0	0.0
9	61.9	10.7	14.5	14.1	11.7	13.3	13.3	13.3	13.3	13.5	16.4	12.0
10	59.5	10.7	5.3	5.4	0.0	4.5	4.5	4.5	4.5	4.8	13.6	7.2

APPENDIX B: FINAL DATA.

The final pH, Chemstor acidity and mbisture content, and length of storage for each unit are presented in the following pages.

Each section is headed by the Moisture level, Chemstor application level and Replicate number. The Moisture and Chemstor levels correspond to the obtained initial experimental levels, which are given in table 11.

The columns in each section appropriately identified, correspond to, respectively, pH, Chemstor acidity, Moisture content and Storage time.

The rows in each section (numbered one to ten) correspond to the imposed experimental conditions. Reference may be made to table 8 for the row identification. The pH data is given in absolute values; Chemstor acidity as percent; Moisture content as percent wet weight weight; and Storage time in days.

Unit	pH	Chemstor acidity		Moisture Content	Storage Time
	Moisture 1		Chemstor level 1	Replicate 1	
1	5.6	0.08		19.8	40
2	4.8	0.39		19.6	150
3	5.6	0.08		19.8	50
4	4.8	0.40		19.5	150
5	5.5	0.11		19.5	50
6	5.4	0.13		19.4	60
7	4.8	0.39		19.2	150
8	5.6	0.06		19.7	60
9	5.3	0.10		20.0	50
10	5.5	0.07		19.8	50
	Moisture 1		Chemstor level 2	Replicate 1	
1	5.0	0.30		19.9	150
2	4.6	0.53		20.0	150
3	5.1	0.27		20.1	150
4	4.6	0.52		19.7	150
5	5.1	0.28		19.7	150
6	5.1	0.29		19.7	150
7	4.6	0.45		19.8	150
8	5.0	0.34		20.1	150
9	4.7	0.46		19.7	150
10	5.0	0.29		19.2	150
	Moisture 1		Chemstor level 3	Replicate 2	
1	4.9	0.37		19.5	150
2	4.7	0.40		19.1	150
3	4.9	0.37		19.3	150
4	4.7	0.44		19.0	150
5	5.1	0.30		19.0	150
6	5.0	0.34		18.8	150
7	4.7	0.45		19.1	150
8	5.1	0.34		19.1	150
9	4.8	0.41		18.7	150
10	5.0	0.34		19.5	150

Unit	pH	Chemstor acidity	Moisture Content	Storage Time
	Moisture 1	Chemstor level 1	Replicate 2	
1	5.3	0.12	18.3	50
2	4.9	0.34	18.6	150
3	5.3	0.12	18.2	50
4	4.8	0.39	18.6	150
5	5.3	0.54	17.9	150
6	5.3	0.17	18.5	150
7	4.9	0.28	18.1	150
8	5.2	0.20	18.0	150
9	5.0	0.17	18.4	50
10	5.3	0.07	17.8	50
	Moisture 1	Chemstor level 2	Replicate 2	
1	5.0	0.24	17.8	150
2	4.8	0.37	18.0	150
3	5.1	0.20	18.1	150
4	4.7	0.42	17.6	150
5	5.0	0.28	17.8	150
6	5.0	0.29	18.9	150
7	4.7	0.39	17.9	150
8	5.0	0.29	17.9	150
9	4.7	0.38	18.6	150
10	5.0	0.24	18.0	150
	Moisture 1	Chemstor level 3	Replicate 2	
1	5.0	0.31	18.4	150
2	4.6	0.51	18.3	150
3	4.9	0.33	18.5	150
4	4.6	0.48	17.6	150
5	5.0	0.37	18.7	150
6	4.9	0.32	18.6	150
7	4.6	0.48	17.9	150
8	4.9	0.35	18.3	150
9	4.7	0.43	18.6	150
10	4.9	0.37	18.6	150

Unit	pH	Chemstor acidity	Moisture Content	Storage Time
	Moisture 2	Chemstor level 1	Replicate 1	
1	5.6	0.15	23.3	50
2	4.8	0.43	23.0	150
3	5.5	0.11	23.2	40
4	4.8	0.50	22.6	150
5	5.4	0.14	22.9	60
6	5.1	0.20	23.3	150
7	4.8	0.49	23.3	150
8	5.1	0.19	22.9	150
9	4.8	0.49	23.3	150
10	5.3	0.13	23.4	40
	Moisture 2	Chemstor level 2	Replicate 1	
1	4.8	0.34	23.4	150
2	4.6	0.64	23.2	150
3	4.8	0.35	22.3	150
4	4.6	0.66	23.2	150
5	4.9	0.32	21.9	150
6	4.9	0.33	23.3	150
7	4.6	0.56	22.8	150
8	4.9	0.34	23.3	150
9	4.6	0.57	22.8	150
10	4.8	0.30	22.2	150
	Moisture 2	Chemstor level 3	Replicate 1	
1	5.3	0.28	23.5	110
2	4.5	0.64	22.6	150
3	4.8	0.46	22.7	150
4	4.5	0.70	23.3	150
5	4.8	0.39	22.3	150
6	4.8	0.39	22.8	150
7	4.5	0.68	22.8	150
8	4.9	0.41	22.9	150
9	4.5	0.64	22.3	150
10	5.3	0.24	23.2	100

Unit	pH	Chemstor acidity	Moisture Content	Storage Time
	Moisture 2	Chemstor level 1	Replicate 2	
1	5.0	0.18	22.0	60
2	4.6	0.43	22.1	150
3	5.3	0.18	21.7	50
4	4.8	0.43	21.5	150
5	5.3	0.19	22.2	110
6	5.0	0.24	22.0	150
7	4.9	0.45	21.5	150
8	5.1	0.24	22.3	150
9	4.8	0.42	22.3	150
10	5.1	0.21	22.0	60
	Moisture 2	Chemstor level 2	Replicate 2	
1	5.0	0.26	21.5	150
2	4.7	0.49	21.8	150
3	4.9	0.29	22.1	150
4	4.7	0.49	22.0	150
5	5.1	0.25	21.4	150
6	5.0	0.29	22.2	150
7	4.7	0.47	22.0	150
8	5.0	0.28	22.1	150
9	4.7	0.47	22.2	150
10	4.8	0.35	21.8	150
	Moisture 2	Chemstor level 3	Replicate 2	
1	4.8	0.40	22.2	150
2	4.6	0.56	21.2	150
3	4.9	0.35	21.6	150
4	4.6	0.56	22.4	150
5	5.0	0.34	22.8	150
6	5.0	0.37	21.8	150
7	4.6	0.56	22.4	150
8	5.0	0.40	22.0	150
9	4.6	0.54	21.1	150
10	4.8	0.40	22.3	150

Unit	pH	Chemstor acidity	Moisture Content	Storage Time
	Moisture 3	Chemstor level 1	Replicate 1	
1	5.5	0.20	25.3	40
2	4.5	0.63	24.7	150
3	5.4	0.21	25.3	40
4	4.6	0.63	25.1	150
5	5.6	0.12	25.0	50
6	5.2	0.23	25.4	150
7	4.6	0.58	24.6	150
8	5.2	0.24	25.5	150
9	4.6	0.55	24.5	150
10	5.3	0.18	25.4	50
	Moisture 3	Chemstor level 2	Replicate 1	
1	4.9	0.53	25.5	150
2	4.5	0.79	25.4	150
3	4.8	0.53	24.7	150
4	4.5	0.81	25.1	150
5	4.9	0.42	24.9	150
6	4.9	0.42	25.6	150
7	4.5	0.81	25.3	150
8	4.9	0.47	25.4	150
9	4.5	0.80	25.4	150
10	4.8	0.58	25.1	150
	Moisture 3	Chemstor level 3	Replicate 1	
1	4.7	0.60	25.1	150
2	4.5	0.87	24.9	150
3	4.7	0.60	24.9	150
4	4.5	0.83	25.0	150
5	4.8	0.51	25.1	150
6	4.8	0.58	24.8	150
7	4.5	0.85	24.4	150
8	4.8	0.53	25.1	150
9	4.5	0.81	25.4	150
10	4.7	0.60	24.9	150

Unit	pH	Chemstor acidity	Moisture Content	Storage Time
	Moisture 3	Chemstor level 1	Replicate 2	
1	5.5	0.17	25.5	40
2	5.2	0.20	26.1	60
3	5.3	0.25	25.5	40
4	4.7	0.55	24.9	150
5	5.5	0.14	25.9	50
6	5.2	0.21	25.6	150
7	4.7	0.51	25.0	150
8	5.1	0.23	25.1	150
9	4.7	0.21	25.8	150
10	5.1	0.34	25.4	50
	Moisture 3	Chemstor level 2	Replicate 2	
1	4.8	0.50	25.0	150
2	4.5	0.76	25.4	150
3	5.2	0.30	25.7	60
4	4.6	0.77	25.8	150
5	4.9	0.34	25.1	150
6	5.1	0.35	25.9	150
7	4.5	0.77	26.0	150
8	4.9	0.47	25.6	150
9	4.5	0.77	25.7	150
10	5.0	0.50	25.4	50
	Moisture 3	Chemstor level 3	Replicate 2	
1	4.8	0.60	25.6	150
2	4.5	1.01	25.7	150
3	4.7	0.67	26.0	150
4	4.5	0.94	25.8	150
5	4.8	0.51	25.8	150
6	4.8	0.62	25.9	150
7	4.5	0.93	25.8	150
8	4.7	0.06	25.3	150
9	4.5	0.92	25.8	150
10	4.7	0.67	25.2	150

APPENDIX C: MICROBIAL ANALYSIS

The microbial counts and mould descriptions for each unit are presented in the following pages.

Each section is headed by the Moisture level, Chemstor application level and Replicate number. The Moisture and Chemstor levels correspond to the obtained initial experimental levels, which are given in table 11.

The columns in each section correspond to, respectively, unit number, microbial analysis intervals, bacterial count, mould count and mould description.

The units (numbered one to ten) are subdivided into 50, 100 and 150 day microbial analysis intervals. Reference may be made to table 8 for unit identification. Only counts greater than 300 are recorded. Counts less than 300 are represented with asterisks. Zeros signify the absence of microflora. Units removed from the experimental conditions are represented by dashes. The counts are given as per gram of grain.

A. gl., A. Ruber, A. Fl. and Pen. represent, respectively, *Aspergillus glaucus*, *Aspergillus ruber*, *Aspergillus flavus* and *Penicillium*.

	Moisture 1 Chemstor level 1 Replicate 1			Moisture 1 Chemstor level 2 Replicate 1			Mojsture 1 Chemstor level 3 Replicate 1		
	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description
1 50	**	270000	12% A.gl., 13% A. ruber, 75% A.fl.	**	**		**	310	35% A.fl.,65%A. ruber
100	-	-		**	0		**	**	
150	-	-		**	0		**	0	
2 50	**	0		**	0		**	**	
100	**	0		**	0		**	0	
150	**	0		**	0		**	0	
3 50	2800	12700	17%A.gl., 83% A. fl.	**	**		**	330	20%A.gl.,40%A.ruber,40%A.fl
100	-	-		**	0		**	**	& mucor
150	-	-		**	0		**	0	
4 50	**	0		**	0		**	0	
100	**	0		**	0		**	0	
150	**	0		**	0		**	0	
5 50	160000	4500	2% A. ruber,8% A.fl.,90%A.gl.	**	420	8%A.ruber,37%A.gl.,55%A.fl.	**	710	18%A.fl.,27%A.ruber,55%A.gl
100	-	-		**	480	7%A.fl.,9%C.h.,17%A.ruber,67%A.gl.	360	900	7%A.fl.,18%A.ruber,75%A.gl
150	-	-		**	**		**	550	& pen. & mucor 8%A.fl.,19%A.gl.,73%A.ruber
6 50	**	3200	3%A,ruber,97%A.gl.	**	300	not identified	**	360	28%A.fl.,28%A.gl., 44%A.ru-
100	-	-		430	**		**	380	ber + mucor 7%A.gl.,35%A.fl.,58%A.ruber
150	-	-		3010	**		**	**	
7 50	**	0		**	0		**	0	
100	**	0		**	0		**	0	
150	**	0		**	0		**	0	
8 50	610	2600	5%A.fl., 95%A.Gl.	**	**		**	480	2%A.gl.,28%A.fl.,70%A.ruber
100	-	-		**	**		**	410	5%A.gl.,33%A.fl.,62%A.ruber
150	-	-		**	**		**	**	
9 50	6300	14700	not identified	**	0		**	0	
100	-	-		**	0		**	0	
150	-	-		**	0		**	0	
10 50	620	147000	33%A.fl.,67%A.gl	**	**		**	**	
100	-	-		**	0		**	**	
150	-	-		**	0		**	**	

e - cladsporium herbarum

		Moisture 1 Chemstor level 1 Replicate 2			Moisture 1 Chemstor level 2 Replicate 2			Moisture 1 Chemstor level 3 Replicate 2		
		Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description
1	50	1900	440	not identified	**	490	1%A.gl.,29%A.fl.,70%A.ruber	**	630	18%A.fl.,82%A.ruber
	100	-	-		**	**		**	**	
	150	-	-		**	**		**	**	
2	50	**	0		0	0		0	0	
	100	**	0		**	0		**	0	
	150	**	0		**	0		0	0	
3	50	8400	6900	not identified	**	530	26%A.fl.,74%A. ruber	**	480	8%A.gl.,8%A.fl.,84%A.ruber & micor
	100	-	-		**	**		**	**	
	150	-	-		**	**		**	**	
4	50	0	0		0	0		0	0	
	100	**	0		**	0		**	0	
	150	**	0		**	0		**	0	
5	50	97000	6710	1%A.fl.,4%Pen.,18%ruber,77%A.gl.	13600	7000	not identified	1960	1410	19%A.fl.,35%A.ruber,46%A.gl., & mucor
	100	13100	4700	7%A.ruber,93%A.fl., + mucor	3820	700	16%A.fl.,24%a.gl.,60%A.ruber & mucor	2910	490	4%A.gl.,25%A.fl.,71%A.ruber & mucor
	150	6500	4000	25%A.gl.,75%A.ruber	1020	520	6%A.gl.,30%A.fl.,64%A.ruber & mucor	**	350	9%A.gl.,24%A.fl.,67%A.ruber
6	50	2500	4080	100%A.gl. & mucor	750	6430	2%A.gl.,98%a.ruber & mucor	**	500	6%A.gl.,25%A.fl.,70%A.ruber & mucor
	100	4830	1090	10%A.ruber,90%A.gl., & mucor	**	430	4%A.gl.,15%A.fl.,81%A.ruber & mucor	**	400	37%A.fl.,63%A.ruber & mucor
	150	15000	1100	not identified	**	310	25%A.fl.,75% A.ruber	**	440	23%A.fl.,77%A.ruber
7	50	0	0		**	0		0	0	
	100	**	0		**	0		**	0	
	150	**	0		0	0		**	0	
8	50	**	1340	100% A.fl. & mucor	**	470	10%A.gl.,19%A.fl.,71%A.ruber	**	460	40%A.fl.,60%A.ruber
	100	**	620	3%A.fl.,30%A.ruber,67%A.gl., & mucor	4500	500	3%A.gl.,19%A.fl.,78%A.ruber & mucor	**	380	25%A.fl.,75%A.ruber
	150	**	620	6%A.fl.,24%A.ruber,80%A.gl.	**	330	40%A.fl.,60%A.ruber&mucor	**	350	27%A.fl.,73%A.ruber & mucor
9	50	**	0		**	0		0	0	
	100	-	-		**	0		**	0	
	150	-	-		**	0		**	0	
10	50	5000	79000	100%A.fl.	**	450	20%A.fl.,76%A.ruber	**	370	21%A.fl.,79%A.ruber
	100	-	-		**			**	**	
	150	-	-		**			**	**	

		Moisture 2 Chemstor level 2 Replicate 1			Moisture 2 Chemstor level 2 Replicate 1			Moisture 2 Chemstor level 3 Replicate 1		
		Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description
1	50	0	17600	8% Pen.,16%A.gl.,76%A.fl.	**	**		**	**	
	100	-	-		**	0		**	**	
	150	-	-		**	0		-	-	
2	50	**	0		0	0		**	0	
	100	**	0		**	0		**	**	
	150	**	0		0	0		0	0	
3	50	**	225000	6%A.gl.,94%A.fl.	**	**		**	**	
	100	-	-		**	0		**	**	
	150	-	-		**	0		**	**	
4	50	0	0		**	0		**	0	
	100	0	0		**	0		**	0	
	150	0	0		0	0		**	0	
5	50	78	1350	4% Pen.,16%A.ruber,80%A.gl.,&mucor	**	**		**	460	12%A.gl.,30%A.fl.,58%A.ruber
	100	-	-		**	4000	26%A.ruber,74%A.fl.	**	380	not identified
	150	-	-		**	**		**	300	15%A.gl.,27%A.fl.,58%A.ruber
6	50	**	530	5%A.fl.,14%A.ruber,80%A.gl.	**	0		**	300	40%A.fl.,60%A.ruber
	100	**	**		**	**		**	**	
	150	**	**		**	**		**	**	
7	50	**	0		**	0		**	0	
	100	**	0		0	0		**	0	
	150	**	0		0	0		**	0	
8	50	**	500	not identified	300	**		**	**	
	100	**	**		**	**		**	**	
	150	**	**		**	**		**	**	
9	50	**	0		0	0		**	0	
	100	**	0		**	0		**	0	
	150	**	0		**	0		**	**	
10	50	300	480000	100%A.fl	**	0		**	**	
	100	-			**	0		-	-	
	150	-			**	0				

		Moisture 2 Chemstor level 1 Replicate 2			Moisture 2 Chemstor level 2 Replicate 2			Moisture 2 Chemstor level 3 Replicate 2		
		Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description
1	50	**	10000	not identified	**	**		**	**	
	100	-	-		**	0		**	**	
	150	-	-		**	0		**	0	
2	50	0	0		0	0		0	0	
	100	**	0		0	0		**	0	
	150	0	0		**	0		0	0	
3	50	**	13000	4%A.gl.,96%A.fl.	**	**		**	400	13%A.gl.,28%A.fl.,59%A.ruber
	100	-	-		**	0		**	**	
	150	-	-		**	0		**	**	
4	50	0	0		0	0		0	0	
	100	**	0		0	0		**	0	
	150	**	0		**	0		0	0	
5	50	**	1100	7%A.fl.,93%A.gl.	**	540	14%A.fl.,21%A.gl.,65%A.ruber	**	670	27%A.fl.,73%A.ruber
	100	**	460	6%A.fl.,44%A.ruber,50%A.gl.	**	410	15%A.fl.,30%A.gl.,55%A.ruber	**	600	27%A.fl.,73%A.ruber
	150	-	-		**	330	13%A.gl.,20%A.fl.,67%A.ruber&mucor	**	480	22%A.fl.,78%A.ruber
6	50	**	390	38%A.fl.,10%A.ruber,52%A.gl.	**	510	4%A.gl.,16%A.fl.,80%A.ruber	**	590	23%A.fl.,77%A.ruber
	100	**	**		**	**		**	**	
	150	**	**		**	**		**	**	
7	50	0	0		0	0		0	0	
	100	**	0		0	0		**	0	
	150	0	0		**	0		0	0	
8	50	**	440	7%A.fl.,40%A.ruber,43%A.gl.	**	**		910	420	11%A.gl.,35%A.fl.,44%A.ruber
	100	**	**		**	**		**	**	
	150	**	**		**	**		**	**	
9	50	0	0		0	0		0	0	
	100	**	0		0	0		**	**	
	150	0	0		0	0		**	0	
10	50	**	0		**	**		**	**	
	100	-	-		**	0		**	0	
	150	-	-		**	0		**	0	

	Moisture 3 Chemstor level 1 Replicate 1			Moisture 3 Chemstor level 2 Replicate 1			Moisture 3 Chemstor level 3 Replicate 1		
	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description
1 50	**	457000	7%A.ruber,93%A.gl.	**	**		0	**	
100	-	-		**	**		**	**	
150	-	-		**	0		**	0	
2 50	**	0		0	0		**	0	
100	**	0		0	0		0	0	
150	0	**		0	0		0	0	
3 50	60	1800000	100%A.gl.	**	**		**	**	
100	-	-		**	**		**	**	
150	-	-		**	0		**	0	
4 50	**	0		**	0		0	0	
100	**	0		**	0		0	0	
150	0	0		**	0		0	0	
5 50	**	890	20%A.ruber,16%A.fl.,82%A.gl.	**	570	not identified	**	1810	3%A.ruber,4%A.gl.,93%A.fl.
100	-	-		**	570	5%A.gl.,28%A.ruber,67%A.fl.,&mucor	**	350	37%A.fl.,63%A.ruber
150	-	-		**	340	8%A.gl.,23%A.fl.,69%A.ruber	**	320	2%A.gl.,29%A.fl.,69%A.ruber
6 50	**	700	not identified	0	440	25%A.fl.,27%A.gl.,48%A.ruber	0	380	39%A.fl.,61%A.ruber
100	**	**		**	**		**	**	
150	2960	**		**	**		**	**	
7 50	**	0		**	0		0	0	
100	0	0		0	0		0	0	
150	0	**		0	0		0	0	
8 50	**	**		**	400	3% Pen.,34%A.ruber,63%A.fl	0	370	10%A.gl.,30%A.ruber,60%A.fl.
100	**	**		0	**		**	**	
150	**	**		**	**		**	**	
9 50	**	0		**	0		**	0	
100	**	0		0	0		0	0	
150	**	0		**	0		**	0	
10 50	**	4300	mycelium only	**	**		**	**	
100	-	-		**	0		**	0	
150	-	-		**	0		**	**	

		Moisture 3 Chemstor level 1 Replicate 2			Moisture 3 Chemstor level 2 Replicate 2			Moisture 3 Chemstor level 3 Replicate 2		
		Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description	Bacteria	Mould	Mould Description
1	50	300	590000	1% Pen.,2%A.ruber,48%A.gl.,49%A.fl.	**	**		**	**	
	100	-	-		**	**		**	0	
	150	-	-		0	0		**	0	
2	50	1130	-		0	0		**	0	
	100	-	-		0	0		0	0	
	150	-	-		**	0		0	0	
3	50	**	420000	20%A.fl.,80%A.gl.	**	**		**	**	
	100	-	-		-	-		**	**	
	150	-	-		-	-		**	0	
4	50	**	0		**	0		**	0	
	100	**	0		0	0		**	0	
	150	0	0		0	0		0	0	
5	50	**	65000	not identified	**	510	5%A.ruber,95%A.fl.	**	2000	not identified
	100	-	-		**	510	10%A.gl.,40%A.fl.,50%A.ruber	**	310	45% A.ruber,55%A.fl&mucor
	150	-	-		**	**		**	**	
6	50	**	2180	8%A.fl.,25%A.ruber,67%A.gl.	**	610	17%A.ruber,12%A.fl.,71%A.gl.	**	460	10%A.fl.,90%A.ruber&mucor
	100	**	300	1%A.ruber,99%A.gl.	**	**		**	**	
	150	340	**		**	49700	100%A.gl.	**	0	
7	50	**	0		**	0		**	0	
	100	**	0		0	0		0	0	
	150	**	0		0	0		**	0	
8	50	**	**		**	**		**	**	
	100	6400	**		**	**		**	**	
	150	**	**		**	**		**	**	
9	50	**	0		**	0		**	0	
	100	**	0		0	0		0	0	
	150	**	0		0	0		0	0	
10	50	**	**		**	**		**	**	
	100	-	-		-	-		**	0	
	150	-	-		-	-		**	0	

APPENDIX D: DUNCAN'S TEST.

Duncan's Tests are presented in the following pages. The data tested is approximately identified. The experimental levels corresponding to the obtained mean values are arranged in an ascending order of magnitude. Reference may be made to tables 12, 61 and 20, respectively, for Duncan's Tests a, b and c, and d.

The mean values underscored by the same line are not significantly different at the 0.95 probability level.

For Duncan's Test (a), reference may be made to section 6.1 regarding the method of underscoring.

(a) Duncan's Test - mean CO_2 production for each temperature condition.

Days										
10	3	1	4	2	5	7	9	6	8	10
20	6	3	4	5	8	9	10	7	1	2
30	8	7	6	2	5	10	9	1	3	4
40	8	6	7	9	2	4	5	10	1	3
50	8	6	4	2	5	7	9	10	1	3
60	8	6	1	10	3	5	7	2	9	4
70	8	3	6	10	1	5	9	2	4	7
80	8	3	6	10	1	5	2	4	7	9
90	8	3	6	10	1	5	2	7	4	9
100	8	3	6	1	5	10	2	7	4	9
125	8	6	5	3	9	2	7	4	1	10
150	8	6	1	10	5	9	3	4	2	7

(b) Duncan's Test - final mean Chemstor acidity due to temperature

5	3	1	6	10	8	9	2	4	7

(c) Duncan's Test - Chemstor application rate within each moisture content.

Initial moisture content			
18.7	1	2	3
22.5	1	2	3
25.5	1	2	3

(d) Duncan's Test - length of storage

(i) - Chemstor application rate within moisture

M_1	1	2	3
M_2	1	3	2
M_3	1	2	3

(ii) Temperature

1	10	3	5	9	2	6	8	4	7